# Session 2A

# New Compounds, New Concepts, New Uses and New Approaches 1

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## Orysastrobin: critical design features for a rice fungicide

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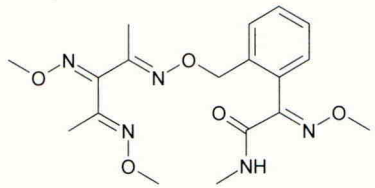
**Abstract**
Orysastrobin is a new rice fungicide that was developed by BASF Aktiengesellschaft and has recently been introduced in the Japanese and Korean markets. It is highly effective against the major rice diseases leaf and panicle blast (*Magnaporthe oryzae,* conidial state *Pyricularia oryzae*) and sheath blight *(Thanatephorus cucumeris,* mycelial state *Rhizoctonia solani*). Orysastrobin has protective, curative, translaminar and systemic properties and thus, a broad and flexible application window. Orysastrobin is characterised by excellent crop safety, has a favourable toxicological and ecotoxicological profile and is hence, safe to users and the environment.

**Introduction**
Orysastrobin is, after the successful launches of kresoxim-methyl, pyraclostrobin and dimoxystrobin (co-development with Shionogi), BASF's fourth active ingredient from the class of strobilurins that has been introduced in the market. Like its predecessor molecules from this class, orysastrobin's biochemical target is the $bc_1$ complex in mitochondrial respiration. Due to its unique biological profile, orysastrobin fills the activity and application gap of BASF's three other strobilurin fungicides and thus, allows for broad application in rice. This paper describes orysastrobin's discovery, its chemical and physical properties, its toxicological and ecotoxicological profile, and its fungicidal efficacy.

**Discovery**
In the mid 1990s BASF had set up a dedicated optimisation program in strobilurin chemistry targeting the two major fungal rice pathogens rice blast and sheath blight. The challenge consisted in the paradigm shift from foliar application to root uptake and how to deal with this issue in a scientific way. By targeted stepwise alterations of the strobilurin core structure, it was found that optimisation analogues with a lower $\log P_{OW}$ and significantly higher water solubility than kresoxim-methyl, pyraclostrobin and dimoxystrobin have the prerequisite to be powerful rice fungicides with excellent root uptake behaviour. Furthermore, this rational adjustment of physicochemical properties went hand in hand with an improved aquatic toxicological profile. Eventually, a novel and structurally unique strobilurin containing four stereospecific oximether functions, namely orysastrobin, was discovered.

The compound was subsequently developed and gained its first global approval in Japan in 2006, ultimately allowing for a successful market launch in 2007. Four formulations were developed for use in seedling boxes or for water surface application, including combinations with the insecticide fipronil. Orysastrobin and its mixtures are being sold under the trade name Arashi.

## Chemical and physical properties

| | |
|---|---|
| CAS number | *[248593-16-0]* |
| Chemical name (IUPAC) | (2*E*)-2-(methoxyimino)-2-{2-[(3*E*,5*E*,6*E*)-5-(methoxyimino)-4,6-dimethyl-2,8-dioxa-3,7-diazanona-3,6-dien-1-yl]phenyl}-*N*-methylacetamide |
| Structural formula |  |
| Molecular formula | $C_{18}H_{25}N_5O_5$ |
| Molecular weight | 391.43 g $mol^{-1}$ |
| Melting point | 99 °C |
| Vapour pressure | $7 \times 10^{-7}$ Pa (20 °C) |
| Partition coefficient *n*-octanol/water | $logP_{OW} = 2.36$ |
| Solubility in water | 80.6 mg/L (20 °C) |

## Product safety

### Mammalian toxicity

| | | |
|---|---|---|
| Acute oral $LD_{50}$ | Rat | 356 mg/kg |
| Acute dermal $LD_{50}$ | Rat | > 2000 mg/kg |
| Eye irritation | Rabbit | no irritation |
| Skin irritation | Rabbit | no irritation |
| Skin sensitisation | Guinea pig | no sensitisation |
| Inhalation, dust $LD_{50}$ | Rat | 2.02 mg/L |

### Wildlife toxicity

| | | |
|---|---|---|
| Bird | *Colinus virginianus* | $LD_{50}$ > 2000 mg/kg |
| Fish | Rainbow trout | $LC_{50}$ = 0.89 mg/L (96h) |
| Daphnia | *Daphnia magna* | $LC_{50}$ = 1.3 mg/L (24h) |
| Earthworm | *Eisenia fetida* | $LC_{50}$ > 1000 mg/kg |
| Bees | Honey bee (adult) | NOEC > 142 µg a.i./bee |
| Algae | Green algae (*Selenastrum capricornutum*) | $EbC_{50}$ (0~72h) = 7.1 mg/L |

### Environmental fate

| | | |
|---|---|---|
| Hydrolysis in water | $DT_{50}$ | > 365 days |
| Photolysis in water | $DT_{50}$ | 0.8 days (natural water, irradiated) |
| Degradation in soil | $DT_{50}$ | 51-58 days (field) |
| Mobility in soil | $K_{OC}$ | 17.9-146 |

**Biological profile**
Biochemically, orysastrobin acts like all strobilurins as an inhibitor of the cytochrome $bc_1$ complex at the Qo site in mitochondrial respiration. In a yeast electron transport particle preparation, the rate of ubihydroquinone:cytochrome-c oxidoreductase was inhibited by 50% by $2.5 \times 10^{-7}$ mol/L orysastrobin in comparison with the untreated control.

Orysastrobin is characterised by outstanding long-lasting disease control due to its strong inhibition of spore germination. Furthermore, the compound is able to suppress mycelial growth reliably, ultimately resulting in very good curative efficacy. The excellent root uptake and translocation behaviour of orysastrobin builds the basis for successful applications in seedling boxes and paddy fields. Distinct acropetal transport in the leaves as well as translaminar activity are additional benefits of the compound.

Orysastrobin is furthermore characterised by excellent crop safety in a broad range of rice varieties. At the recommended rates, no crop injuries have been observed either in seedling boxes or after water surface applications.

**Fungicidal efficacy**

**Method** (Stammler *et al.*, 2007)
Fungicides were applied as granular formulations to seedling boxes. Each fungicide (50 g) was scattered in each seedling box (30 × 60 cm), corresponding to 10 kg product/ha. Applications were made on the day of transplanting. For trials investigating the influence of application timing on selectivity and efficacy, applications were made at the seedling stage (after seeding, before soil covering), at the greening stage (2 days after emergence) and on the day of transplanting.

Seedlings were transplanted into paddy fields using a commercial mechanical transplanter (Kubota, two rows). Trials were performed in 2002 and 2003 at different trial sites in Japan. In each trial, three randomised replicates per treatment were used with a minimum plot size of 15 $m^2$. The cultivation of the crop was according to normal practical standards. The rice variety used was Koshihikari.

Leaf blast was evaluated at growth stage (GS) 24-34, which corresponds to 48-64 days after transplanting. Panicle blast was evaluated at GS 77-85, which corresponds to 85-103 days after transplanting. Sheath blight was assessed at GS 77-87, corresponding to 86-106 days after transplanting.

**Results** (Stammler *et al.*, 2007)
In all trials, orysastrobin provided excellent control of leaf and panicle blast, equal or superior to standard commercial products. Furthermore, orysastrobin showed outstanding, long-lasting disease control for more than 100 days after application and its efficacy was independent of application timing and disease pressure. (Tables 1-3)

Table 1. Efficacy of orysastrobin against leaf blast (trial location Tahara, Aichi, 2002)

| Product | Application rate (g a.i./ha) | Trial 1 % control (5.0% in untreated) | Trial 2 % control (14.3% in untreated) |
|---|---|---|---|
| Orysastrobin | 700 | 99 | 98 |
| Orysastrobin + Fipronil | 700 | 99 | 98 |
| Orysastrobin + Clothianidin | 700 | 99 | 99 |
| Dicyclomet combination | 300 | 94 | 95 |
| Probenazole combination | 2400 | 99 | 95 |
| Pyroquilon combination | 1200 | 91 | 98 |

Table 2. Efficacy of orysastrobin against panicle blast (trial location Tahara, Aichi, 2002)

| Product | Application rate (g a.i./ha) | Trial 1 % control (3.0% in untreated) | Trial 2 % control (12.5% in untreated) |
|---|---|---|---|
| Orysastrobin | 700 | 92 | 97 |
| Orysastrobin + Fipronil | 700 | 94 | 97 |
| Orysastrobin + Clothianidin | 700 | 91 | 98 |
| Dicyclomet combination | 300 | 80 | 90 |
| Probenazole combination | 2400 | 67 | 79 |
| Pyroquilon combination | 1200 | 67 | 81 |

Table 3. Influence of application timing on the control of leaf and panicle blast (trial location Tahara, Aichi, 2003)

| Product | Application timing | Leaf blast % control (3.0% in untreated) | Panicle blast % control (4.2% in untreated) |
|---|---|---|---|
| Orysastrobin + | Seeding | 97 | 97 |
| Fipronil (700 g | Greening | 96 | 94 |
| a.i./ha) | Transplanting | 96 | 96 |
| Dicyclomet | Seeding | 83 | 82 |
| combination (300 g | Greening | 87 | 79 |
| a.i./ha) | Transplanting | 84 | 77 |

In addition, orysastrobin provided, again independently from application timing and disease pressure, excellent control of sheath blight, comparable to standard market products. (Tables 4 and 5)

Table 4. Efficacy of orysastrobin against leaf blast (trial location Tahara, Aichi, 2002)

| Product | Application rate (g a.i./ha) | Trial 1 % control (7.0% in untreated) | Trial 2 % control (40.2% in untreated) |
|---|---|---|---|
| Orysastrobin | 700 | 95 | 89 |
| Orysastrobin + Fipronil | 700 | 99 | 90 |
| Orysastrobin + Clothianidin | 700 | 97 | 84 |
| Thifluzamide combination | 300 | 52 | 85 |

Table 5. Influence of application timing on the control of sheath blight (trial location Tahara, Aichi, 2003)

| Product | Application timing | % control (6.5% in untreated) |
|---|---|---|
| Orysastrobin + Fipronil (700 g a.i./ha) | Seeding | 100 |
| | Greening | 100 |
| | Transplanting | 98 |
| Furametpyr combination (300 g a.i./ha) | Transplanting | 98 |

**Resistance management**

In order to maintain orysastrobin's outstanding activity, resistance management strategies have been developed (Stammler *et al.*, 2007). The cultivation of less pathogen-susceptible rice varieties and usage of healthy seeds is recommended together with a restriction of the number of applications of strobilurin fungicides. In addition, alternating applications with rice fungicides with a different mode of action, such as dicyclomet, probenazole, pyroquilon, furametpyr, tiadinil or thifluzamide, are recommended.

**Conclusion**

Orysastrobin is BASF's new structurally unique strobilurin fungicide that was specifically designed to target the rice market. It has outstanding long-lasting efficacy on leaf and panicle blast and sheath blight, independent from application timing and disease pressure. Furthermore, orysastrobin shows excellent crop safety. It has a favourable toxicological and ecotoxicological profile and is safe to users and the environment. Four formulations have been developed for use in seedling boxes or for water surface application, including combinations with the insecticide fipronil. These orysastrobin-containing products have been introduced successfully into the Japanese and Korean markets in 2007.

**Acknowledgements**
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**References**

Stammler G; Itoh M; Hino I; Watanabe A; Kojima K; Motoyoshi M; Koch A; Haden E (2007). Efficacy of orysastrobin against blast and sheath blight in transplanted rice. *Journal of Pesticide Science*, **32**, 10-15

# Meptyldinocap: a new active substance for control of powdery mildew

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**Abstract**
Meptyldinocap is a novel contact fungicide with limited penetrant activity providing protectant, curative and eradicant control of powdery mildews. Meptyldinocap is a single isomer of dinocap, offering the benefit of a more favourable toxicological profile. Meptyldinocap acts as an uncoupler of oxidative phosphorylation, upsetting the electrochemical balance of the cell and preventing the formation of energy rich ATP. Its main use will be for control of grape powdery mildew, but other crop uses include cucurbits and strawberries. Due to its unique mode of action amongst powdery mildew fungicides and the fact that no resistance developed after many years of commercial use of dinocap, the resistance risk is judged to be very low. Therefore, meptyldinocap will be extremely useful to the vine growers as a resistance management tool.

## Introduction

Powdery mildew diseases occur on a number of different crops generally causing severe damage leading to substantial yield loss. Their control is a major problem for growers and the use of fungicides is frequently required to protect the crops. However, powdery mildew pathogens present an inherently high risk of developing resistance to fungicides and most of the fungicides currently employed are single site inhibitors, hence increasing the risk.

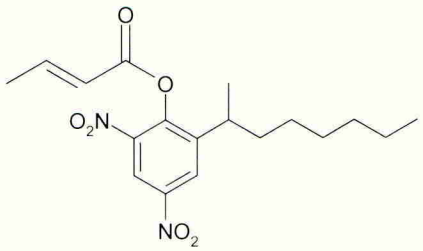
Indeed, powdery mildew resistance to single site fungicides is rather widespread on various crops. As a consequence, use practices need to be adapted in order to prevent potential occurrence or to manage existing resistance. Common recommendations aiming to limit the resistance risk are limited number of applications of single site fungicides per season and use in alternation or mixture with a fungicide of a different mode of action.

Meptyldinocap is a novel fungicide developed by Dow AgroSciences providing protectant, curative and eradicant control of powdery mildew pathogens. Meptyldinocap is a single isomer of dinocap, which has been used for more than 40 years to control powdery mildew diseases. Dinocap benefits from a unique mode of action amongst powdery mildew fungicides, which has proven not to generate any resistance after more than 40 years of use. Therefore, dinocap has become a key element in resistance management programs.

In addition, because of its eradicant efficacy, it is often employed in situations requiring clean-up treatments on established powdery mildew infections. Meptyldinocap benefits from the same mode of action as dinocap and equivalent biological efficacy although presenting a much more favourable toxicological profile. Proposed use patterns in a program, will be similar to those currently recommended for dinocap.

**Chemical and physical properties**

| | |
|---|---|
| DAS code number : | DE-126 |
| Common Name: | Meptyldinocap |
| Chemical Class: | Dinitrophenyl |
| Chemical Name (IUPAC): | 2,4-dinitro-6-(1-methyheptyl)phenyl crotonate |
| Chemical Name (CA): | (*E*)-2-butenoic acid, 2-(1-methylheptyl)-4,6-dinitrophenyl ester |
| CAS number: | *[131-72-6]* |
| Structural formula: | |




| | |
|---|---|
| Empirical Formula: | $C_{18}H_{24}N_2O_6$ |
| Molecular weight: | 364.2 |
| Density: | 1.11 g/cm$^3$ at 20° C |
| Appearance: | Yellow to brown liquid at ambient temperature |
| Melting point: | -22.5° C |
| Boiling point: | Not determined as decomposes at 200° C |
| Water solubility: | 0.248 mg/L at 20° C |
| Vapour pressure: | $7.92 \times 10^{-6}$ Pa at 25° C (non-volatile) |
| Octanol/water partition co-efficient (log $P_{ow}$): | 6.5 at 20° C (pH 7) |

**Mammalian toxicology**

| | | |
|---|---|---|
| Acute oral | Rat | $LD_{50}$ >2000 mg/kg |
| Acute oral | Mouse | $LD_{50}$ >2000 mg/kg |
| Acute dermal | Rat | $LD_{50}$ >5000 mg/kg |
| Dermal irritation | Rabbit | Slightly irritating |
| Eye irritation | Rabbit | Slightly irritating |
| Dermal sensitization | | Positive response in LLNA assay; may cause sensitization by skin contact |
| Mutagenicity | *In vitro/in vivo* | No potential mutagenicity |
| Teratogenicity | Rat, rabbit | No potential teratogenicity |
| Carcinogenicity | Rat, mouse | Not carcinogenic |

Meptyldinocap benefits from a favourable toxicological profile with low acute toxicity. Meptyldinocap is neither teratogenic nor carcinogenic and presents no risk of mutagenic effects.

**Environmental toxicology**

| | | |
|---|---|---|
| Fish acute 96 h $LC_{50}$ | *Oncorhynchus mykiss* Rainbow trout | 0.071 mg/L |
| Fish acute 96 h $LC_{50}$ | *Lepomis macrochirus* Bluegill sunfish | 0.062 mg/L |
| Invertebrate acute 48 h $EC_{50}$ | *Daphnia pulicaria* | 0.0041 mg/L |
| Algae $EbC_{50}$ 72 h (biomass) | *Pseudokirchneriella subcapitata* | 4.6 mg/L |
| Acute contact 72 h $LD_{50}$ | Honeybee *Apis mellifera* | 84.8 µg/bee |
| Acute oral 72 h $LD_{50}$ | Honeybee *Apis mellifera* | 90.0 µg/bee |
| Extended laboratory $LR_{50}$ on natural substrate | Predatory mite *Typhlodromus pyri* | 40.7 g as/ha |
| Extended laboratory aged residue on natural substrate-mortality and parasitism rate | Parasitoid wasp *Aphidius rhopalosiphi* | 16.7% mortality at 840 g a.i./ha<br>28.8% effect on parasitism at 840 g a.i./ha |
| Earthworm acute 14 day $LC_{50}$ | *Eisenia foetida* | 302 mg/kg soil |

Meptyldinocap has been shown to be highly toxic to fish and invertebrates and moderately toxic to algae in laboratory studies. However, meptyldinocap is bound tightly to soil which reduces its potential to enter aquatic systems. In addition, any material entering aquatic systems will dissipate rapidly by microbial metabolism, photodegradation and sorption to the sediment. Therefore, when these data are taken together, meptyldinocap is not considered to cause an unacceptable risk to these organisms in surface water.

When tested under a variety of field conditions against several species of beneficial mites, meptyldinocap was always found to have an IOBC classification of Class 1 (harmless) or Class 2 (slightly harmful).

**Fate and behavior in the environment**

| | | |
|---|---|---|
| Soil mobility | Kco | 2889-310200 ml/g (mean 58245 ml/g) |
| Soil degradation | $DT_{50}$ aerobic | 4 - 24 days at 20° C (mean 12 days) |
| | $DT_{50}$ anaerobic | 8 days |
| | $DT_{50}$ field | 15 days |
| Hydrolysis in water | $DT_{50}$ | pH 4: stable<br>pH 7: 31 days<br>pH 9: 9 days |
| Degradation in water | $DT_{50}$ in darkness | 4 - 7 days (mean 6 days) |
| Air | $DT_{50}$ calculated | 1.9 hours |

Meptyldinocap is readily degraded in soil through hydrolysis and microbial degradation

Meptyldinocap is strongly sorbed to soil and the adsorption coefficients indicate that it will have no potential to reach groundwater.

Meptyldinocap will not be persistent in aquatic systems.

Meptyldinocap has a very low vapour pressure and it is therefore considered that significant amounts of meptyldinocap will not be present in air.

**Mode of action**
Biologically, meptyldinocap is active at all stages of the pathogen life cycle. It provides protectant, curative and eradicant control of powdery mildews by inhibiting spore germination, fungal respiration and by causing metabolic disturbances of the fungal cell which result in cell death.

Biochemically, meptyldinocap acts as an uncoupler of oxidative phosphorylation, upsetting the electrochemical balance of the cell and preventing the formation of energy rich ATP.

Since a specific interaction with a biochemical target is not invoked in this mechanism, meptyldinocap can be considered not to act at a single site and, therefore, to be at low risk in terms of resistance management.

Meptyldinocap not only poses a negligible risk of resistance development, its use, in alternation or mixture, with modern single-site fungicides, which are more susceptible to resistance development, diminishes the agronomic risk for these fungicides and preserves their effective life.

**Efficacy**

**Material and method**
Curative efficacy against powdery mildew of grapes (*in vitro*)

One study was carried out by the INRA at Bordeaux in order to evaluate the curative efficacy of meptyldinocap against grape powdery mildew (*Erysiphe necator*). The study was conducted on detached grape leaf discs, maintained in Petri dishes. The leaves were inoculated by dusting the conidia on the upper leaf surface. After inoculation, the leaves were treated at various intervals by spraying the fungicidal solution (2 ml/leaf) on their upper surface using a Potter Burkard spray tower.

Table 1. Fungicides and rates tested (ppm a.i.)

| Fungicide | Rate ppm a.i. |
|---|---|
| Meptyldinocap | 2.1 |
| Dinocap | 2.1 |
| Sulfur | 100 |
| Tebuconazole | 1 |
| Spiroxamine | 3 |
| Trifloxystrobin | 0.625 |

The percentage leaf surface covered with powdery mildew was estimated visually at 6, 8, 10, 13 and 16 days after inoculation. The table of results shows the values of the area under the disease progress curve (AUDPC). In addition, an analysis of variance (ANOVA) followed by the Newman and Keul's means separation test was conducted ($p = 0.05$).

**Field trials**

The field efficacy of meptyldinocap was tested in a series of trials against powdery mildew on grapes, cucurbits (mainly melon) and strawberries. The trials were carried out across Europe according to the relevant EPPO and AFPP-CEB guidelines. A formulation containing 350 g a.i./L of meptyldinocap as an emulsifiable concentrate (EC) was tested under the code number GF-1478 and compared to the reference product dinocap (as Karathane LC 350 g/L) on all crops.

Depending on crops and countries, the use rate of the two products was either 0.6 l/ha (210 g a.i./hl) using water volumes ranging between 100 l/ha and 400 l/ha or 0.06 l/hl (21 g a.i./hl) at water volumes ranging between 400 l/ha and 1000 l/ha depending on the crop growth stages.

The trials were carried out in France, Italy, Spain, Portugal and Hungary. A sulfur based product was included as a reference product in all grape trials at its local registration rate which tends to vary across countries. In Italy, Spain, Portugal and Hungary, a rate of 320 g a.i./hl - 400 g a.i./hl at water volumes varying between 400 – 1000 L/ha was used whereas in France the rate employed was 10 kg a.i./ha throughout the season in all trials.

The trials on cucurbits were carried out in Italy and in Spain on melon and courgettes grown in the open field. A formulation containing 100 g a.i./L of penconazole was used as a reference product in these trials at the local registration rates (4 g a.i./hl in Italy and 3 g a.i./hl in Spain).

The trials on strawberries were conducted in Italy, Spain and in the UK either on strawberries grown in Spanish tunnels or in open field. A formulation containing 45 g a.i./L of myclobutanil was used as the reference product at a use rate of 6 g a.i./hl.

All products were applied season long on a 10 day schedule.

In addition, a series of trials were set up in Italy to evaluate the eradicant efficacy of meptyldinocap on established powdery mildew infections on grape bunches. Meptyldinocap was applied twice at a five day interval at a use rate of 0.06 l/hl using 1000 L/ha of water. The first application was generally carried out around B73 – 75 with infection levels ranging between 10 % - 35 %.

The results presented correspond generally to those obtained at the final observation which was usually conducted at the end of the season (beginning of ripening for the grape trials). An analysis of variance (ANOVA, p = 0.05) was carried out followed by a Newman and Keul's test to separate the means.

**Results**

**Curative efficacy *in vitro* against grape powdery mildew**

Table 2. Curative efficacy *in vitro* of meptyldinocap against grape powdery mildew

| | AUDPC | |
|---|---|---|
| Interval inoculation - treatment | 48 hours | 4 days |
| Untreated | 1176 a | 901 a |
| Dinocap | 1.5 c | 50 b |
| Meptyldinocap | 93 bc | 76 b |
| Sulfur | 28 c | 135 b |
| Tebuconazole | 0 c | 151 b |
| Spiroxamine | 3 c | 42 b |
| Trifloxystrobin | 174 b | 165 b |
| P | < 0.00001 | < 0.00001 |
| CV | 38.8 % | 40.5 % |

numbers followed by different letters are significantly different from each other at P = 0.05

The timing of 48 h after inoculation corresponds typically to the beginning of the mycelium development whereas at 4 days after inoculation the mycelium is usually widespread.

The results confirm the excellent curative activity of meptyldinocap. The efficacy appeared to be marginally weaker than that of tebuconazole, spiroxamine and dinocap in the 48 h curative test, yet the results of the four day curative test indicate a slightly better performance of meptyldinocap than tebuconazole and trifloxystrobin. Nevertheless, the statistical analysis did not reveal any significant differences between the tested products in both studies.

It is worth noting that the study was conducted with a DMI (demethylation inhibitor) sensitive powdery mildew strain. Resistance to DMIs is widespread in European vineyards causing severe loss of efficacy (Steva, 1992). In addition, it is generally preferable to use products presenting a low resistance risk such as meptyldinocap for eradicant treatments and to use single site products mainly in protectant situations in order to preserve their efficacy (Brent, 1995).

**Field efficacy**

Table 3. Efficacy of meptyldinocap against powdery mildew in field trials (2005 – 2006)

| | | Meptyl-dinocap | Dinocap | Sulfur | DMI reference* | Untreated |
|---|---|---|---|---|---|---|
| Grapes, leaves | % Infection | 3 b | 4 b | 10 a | - | 34 |
| (n=14) | (% Efficacy) | (91) | (88) | (71) | - | |
| | % Incidence | 24 b | 27 b | 38 a | - | 76 |
| | (% Efficacy) | (68) | (64) | (50) | - | |
| Grapes, bunches | % Infection | 9 b | 8 b | 19 a | - | 50 |
| (n=25) | (% Efficacy) | (82) | (84) | (62) | - | |
| | % Incidence | 47 b | 43 b | 57 a | - | 83 |
| | (% Efficacy) | (43) | (48) | (31) | - | |
| Grapes, eradicant bunches | % Infection | 17 a | 15 a | - | - | 74 |
| (n = 4) | (% Efficacy) | (71) | (77) | - | - | |
| Cucurbits | % Infection | 28 b | 27 b | - | 39 a | 69 |
| (n=13) | (% Efficacy) | (59) | (61) | - | (43) | |
| Strawberries, leaves | % Infection | 6 a | 7 a | - | 6 a | 31 |
| (n=6) | (% Efficacy) | (80) | (76) | - | (80) | |
| | % Incidence | 38 a | 32 a | - | 29 a | 74 |
| | (% Efficacy) | (54) | (61) | - | (64) | |
| Strawberries, fruit | % Incidence | 15 a | 14 a | - | 18 a | 30 |
| (n=6) | (% Efficacy) | (59) | (62) | - | (52) | |

Numbers followed by different letters are significantly different from each other at P = 0.05

* penconazole on cucurbits, myclobutanil on strawberries

Meptyldinocap provided equally good season long control of powdery mildew on grapes, cucurbits and strawberries as dinocap. In addition, meptyldinocap was equivalent to dinocap when applied twice on a five day interval to clean-up existing powdery mildew infections on bunches.

Furthermore, meptyldinocap demonstrated significantly better control than sulfur and penconazole against powdery mildew on grapes and cucurbits respectively. The poor performance of penconazole on cucurbits is probably due to the presence of DMI resistant strains, leading to considerable loss of efficacy for this type of product. The results show that meptyldinocap is of particular value in such conditions, offering growers an effective solution for resistance management.

**Conclusion**

Meptyldinocap is a novel fungicide which will be of particular interest for resistance management strategies. It can be used in alternation or in mixture with single site fungicides, which are more susceptible to resistance development. Meptyldinocap is an extremely effective curative and eradicant powdery mildewicide.

Therefore, it can be used to stop early and latent infections, keeping inoculum pressure to a minimum and potentially eradicating resistant isolates if they occur at a very low frequency in the population.

Meptyldinocap is safe to beneficial insects such as *T. pyri* and can be successfully adopted in integrated pest management programs offering a disease resistance management tool, excellent powdery mildew control and preservation of the beneficial fauna.

**Acknowledgements**
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# Biological properties of the carboxamide boscalid including recent studies on its mode of action

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## Abstract
Boscalid is a new fungicide belonging to the class of carboxamides with favourable toxicological and ecotoxicological properties. It inhibits spore germination, germ tube elongation and is also effective on all other stages of fungal development. Boscalid is translaminar and acropetally transported within plant leaves. It was first developed for control of a broad range of fungal pathogens in speciality crops, but the compound also exhibits excellent properties for use in arable crops including oilseed rape and cereals. The mixture with epoxiconazole combines the biological properties of both compounds, leading to an effective fungicide in cereals against the relevant pathogens. Sensitivity studies with laboratory mutants and field isolates of *Botrytis cinerea* showed that different mutations in the target gene influence the sensitivity to carboxamides.

## Introduction
Boscalid is the common name of the new fungicide discovered and developed by BASF. It belongs to the carboxamide class of fungicides (SDH-inhibitors). The target enzyme of carboxamides is succinate dehydrogenase (SDH), which is a functional part of the tricarboxylic cycle and mitochondrial electron transport chain (Keon *et al.*, 1991). SDH consists of four subunits (A, B, C and D) and the binding site of ubiquinone (and the site of action of carboxamides) is formed by the subunits B, C and D. The properties of boscalid, its fungicidal profile in speciality crops, the development into arable crops and studies on the sensitivity (monitoring and target site mutation analysis) are described.

## Chemical and physical properties

| | |
|---|---|
| CAS number | *[188425-85-6]* |
| Chemical name (IUPAC) | 2-chloro-*N*-(4′-chlorobiphenyl-2-yl)-nicotinamide |
| Structural formula |  |
| Molecular formula | $C_{18}H_{12}Cl_2N_2O$ |
| Molecular weight | 343.2 |
| Melting point | 142.8-143.8°C |
| Vapour pressure | $7\times10^{-7}$ Pa |
| Partition coefficient n-octanol/water | $LogP_{ow} = 2.96$ |
| Solubility in water (20°C) | 0.00046 g in 100 ml |

## Product safety

### Mammalian toxicity

| | | |
|---|---|---|
| Acute oral $LD_{50}$ | Rat | > 5000 mg/kg |
| Acute dermal $LD_{50}$ | Rat | > 2000 mg/kg |
| Eye irritation | Rabbit | Non-irritating |
| Skin irritation | Rabbit | Non-irritating |
| Skin sensitisation | Guinea pig | Non-sensitizing |
| Mutagenicity | Ames, mouse | Non-mutagenic |
| Teratogenicity | Rat, rabbit | Non-teratogenic |
| Carcinogenicity | Dog, rat, mouse | Non-carcinogenic |
| Reproduction | Rat | No adverse effects |

### Wildlife toxicity

| | | |
|---|---|---|
| Bird (bobwhite quail) | Practically non-toxic | $LD_{50}$ > 2000 mg/kg |
| Earthworm | Practically non-toxic | $LC_{50}$ > 1000 mg/kg |
| Bees | Practically non-toxic | $LD_{50}$ = 100 µg/bee |
| Aquatic organisms | Moderately toxic | |
| Trout | | $LC_{50}$ = 2.7 mg/L |
| Algae | | $LC_{50}$ = 5.3 mg/L |
| Daphnia | | $LC_{50}$ = 3.8 mg/L |

### Biological properties

Microscopical examination of the morphological effects of boscalid on fungal development showed that the compound strongly inhibits spore germination, but is also effective against germ tube development, appressoria formation and mycelial growth (Ammermann *et al.* 2002; Stammler & Speakman, 2006; Stammler *et al.* 2007). Boscalid has excellent preventative efficacy and, depending on pathogen and host, boscalid can also be effective if used curatively. Studies with radiolabelled boscalid showed that it is transported translaminarly and acropetally. Boscalid is safe to plants; therefore it can be used broadly in many different crops at all stages of development.

### Fungicidal profile

**Speciality crops** - In contrast to previously described carboxamide fungicides with main activity against Basidiomycetes, boscalid has a different fungicidal profile. It has been developed in many speciality crops (fruits, grapes, vegetables, ornamentals and turf) against a broad range of fungal pathogens (Deuteromycetes, Ascomycetes and Basidiomycetes). The main target pathogens are *Botrytis* spp., *Alternaria* spp., *Sclerotinia* spp., *Mycosphaerella* spp., *Monilinia* spp. and powdery mildews. Boscalid was launched in some crops as a single compound fungicide.

For different crops and pathogens, combinations with kresoxim-methyl, pyraclostrobin, and others have been developed. These combination products provide an enhanced efficacy combined with broader disease spectrum and contribute to an effective anti-resistance management strategy.

**Cereals and other arable crops** - After first launch in speciality crops, boscalid was developed against different diseases in oilseed rape, peanuts, potatoes and other field crops. For cereals, the combination with epoxiconazole was developed. This co-formulation provides a better curative and long lasting efficacy compared to the performance of the individual active ingredients (a.i.). These different biological properties of both actives lead to a very reliable fungicide for the control of *Mycosphaerella graminicola, Oculimacula* spp. and *Puccinia triticina* in wheat (Table 1).

Table 1. Efficacy of boscalid + epoxiconazole against wheat diseases (% disease)

| Treatment | Rate [g a.i./ha] | Septoria leaf spot (*M.graminicola*) n= 70 | Eyespot (*Oculimacula* spp.) n= 31 | Rust (*P. triticina*) n=19 |
|---|---|---|---|---|
| Untreated | | 54 [a] | 42 [a] | 29 [a] |
| boscalid + epoxiconazole | 350 + 100 | 13 [c] | 15 [c] | 3 [b] |
| Standard (leaf diseases) | 500 | 16 [b] | | 3 [b] |
| Standard (eyespot) | 750 | | 24 [b] | |

Means followed by the same letter do not differ significantly (Student-Newmans Keuls test, $p<0.05$, percentage disease values were arc-sin-square root transformed).

The main target pathogens of this combination in barley are *Pyrenophora teres, Rhynchosporium secalis* and *Puccinia hordei*. *Ramularia collo-cygni*, the new challenge in barley production, is also very well controlled by boscalid + epoxiconazole (Table 2).

Table 2. Efficacy of boscalid + epoxiconazole against barley diseases (% disease)

| Treatment | Rate [g a.i./ha] | Net blotch (*P. teres*) n= 29 | Leaf scald (*R. secalis*) n= 13 | Rust (*P. hordei*) n=11 | Ramularia leaf spot (*R. collo-cygni*) n= 3 |
|---|---|---|---|---|---|
| Untreated | | 49 [a] | 37 [a] | 17 [a] | 24 [a] |
| boscalid + epoxiconazole | 350 + 100 | 15 [c] | 12 [c] | 2 [b] | 4 [c] |
| Standard | 500 | 27 [b] | 14 [b] | 2 [b] | 9 [b] |

Means followed by the same letter do not differ significantly (Student-Newmans Keuls test,

Table 3. Efficacy of boscalid on target pathogens

| Crop | Pathogen | Efficacy |
|---|---|---|
| Cereals | *Oculimacula* spp. | ++++ |
| | *Mycosphaerella graminicola* | +++ |
| | *Pyrenophora teres* | ++++ |
| | *Ramularia collo-cygni* | ++++ |
| | *Rhynchosporium secalis* | +++ |
| Canola | *Sclerotinia sclerotiorum* | ++++ |
| | *Alternaria* spp. | ++++ |
| | *Leptosphaeria maculans* | +++ |
| Grapes | *Botrytis cinerea* | ++++ |
| | *Erysiphe necator* | ++ |
| | *Penicillium* spp. | ++ |
| Fruits | *Monilinia* spp. | ++++ |
| | *Blumeriella jaapii* | ++++ |
| | *Stemphylium vesicarium* | ++++ |
| | *Alternaria* spp. | ++++ |
| | *Sphaerotheca pannosa* | ++ |
| Strawberries | *Botrytis cinerea* | ++++ |
| | *Mycosphaerella fragariae* | +++ |
| | *Sphaerotheca macularis* | ++ |
| Vegetables | *Botrytis cinerea* | ++++ |
| | *Alternaria* spp. | ++++ |
| | *Sclerotinia sclerotiorum* | ++++ |
| | *Sclerotinia minor* | ++++ |
| | *Septoria lycopersici* | +++ |
| | *Ascochyta rabiei* | +++ |
| Ornamentals, turf | *Botrytis elliptica* | ++++ |
| | *Botrytis tulipae, B. gladiolorum* | +++ |
| | *Uromyces dianthi* | +++ |
| | *Diplocarpon roseum* | +++ |
| | *Sphaerotheca pannosa* | ++ |
| | *Puccinia horiana* | ++ |
| | *Sclerotinia homoeocarpa* | ++++ |

(++ moderate, +++ good and ++++ very good efficacy)

**Sensitivity studies**

Isolates of *M. graminicola, Oculimacula* spp., *S. sclerotiorum* and *B. cinerea* from different European countries were tested for their sensitivity to boscalid according to previous published methods (Stammler & Speakman, 2006; Stammler *et al.*, 2007). In 2006, the sensitivity of *M. graminicola, Oculimacula* spp., *S. sclerotiorum* (isolated from beans and oilseed rape) and *B. cinerea* (from grapes and strawberries) towards boscalid was investigated. $ED_{50}$ values from 2006 were compared with the $ED_{50}$ of the baseline. The $ED_{50}$s of all isolates of *M. graminicola*, *Oculimacula* spp. and *S. sclerotiorum* were within the baseline. For *B. cinerea*, more than 1000 isolates were tested Europe-wide and at 3 sites single isolates could be detected with $ED_{50}$ values outside the baseline. These isolates (and mutants generated in the laboratory) showed the mutations P225L/F/T, and H272Y/R in the SDH B-subunit. No mutations occurred in C- or D-subunits. Sequence alignments (Table 4) showed that the position of 272 in *B. cinerea* corresponds to 267 in *M. graminicola* (Skinner *et al.*, 1998), 257 in *Ustilago maydis* (Keon *et al.*, 1991) and 229 in *Xanthomonas campestris* (Li *et al.* 2006). Mutations for the amino acid 225 have so far only been found in *B. cinerea*. P225L, P225F as well as H272Y cause significant losses in fungicide sensitivity, whilst H272R and P225T were less pronounced.

Table 4. Amino acid sequences in the various isolates tested

| | |
|---|---|
| BC (wildtype) | 218ACCSTSCPSYWWNS...SLYRCHTILNCS |
| BC (P225L) | 218ACCSTSC**L**SYWWNS...SLYRCHTILNCS |
| BC (P225F) | 218ACCSTSC**F**SYWWNS...SLYRCHTILNCS |
| BC (P225T) | 218ACCSTSC**T**SYWWNS...SLYRCHTILNCS |
| BC (H272Y) | 218ACCSTSCLSYWWNS...SLYRC**Y**TILNCS |
| BC (H272R) | 218ACCSTSCLSYWWNS...SLYRC**R**TILNCS |
| MG (H267Y) | 213ACCSTSCLSYWWNS...SLYRC**Y**TILNCS |
| UM (H257Y) | 218ACCSTSCLSYWWNQ...KLYRC**L**TILNCA |
| XC (H229Y) | 218ACCSTSCLSYWWNG...KLYRC**Y**TILNCA |

BC (*B. cinerea*), MG (*M. graminicola*), UM (*U. maydis*), XC (*X. campestris*)
Bold letters show mutations conferring resistance to SDH inhibitors

Molecular modeling studies with the crystal structure of SDH from *E. coli* (Yankovskaya *et al.*, 2003) in combination with structural alignments with the sequences of *M. graminicola* and *B. cinerea* provided a three-dimensional model for structural analysis of the identified mutations.

All mutations lie in or close to the ubiquinone-binding site (Q-site). Proline at 225 is part of the Q-site forming hydrophobic contacts to ubiquinone. A mutation of proline into an amino acid with a much bulkier side-chain such as leucine or tyrosine is expected to result in a decreased binding affinity for carboxamides, whereas in the case of the P225T mutation this effect might be smaller as the threonine side-chain is less bulky. A loss in binding affinity can also be expected in the case of the mutations at H272, with its side-chain located at the Q-site, directly involved in the binding of SDH inhibitors via hydrogen-bonding (Horsefield *et al.*, 2006).

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## Cyflumetofen: A novel selective acaricide

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**Abstract**
Cyflumetofen, 2-methoxyethyl (*RS*)-2-(4-*tert*-butylphenyl)-2-cyano-3-oxo-3-(α,α,α-trifluoro-*o*-tolyl)propionate, is a novel acaricide which is highly active against phytophagous mites. It shows excellent efficacy against all developmental stages of mites ($LC_{50}$ < 5 ppm) and no cross-resistance to existing acaricides. It shows a highly selective activity spectrum.

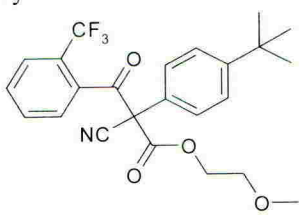
It is extremely effective against *Tetranychus* and *Panonychus* mites, and active against other phytophagous mites, while quite safe to predatory Phytoseiidae mites as well as other non-target organisms. Field trials of 20% SC formulation of cyflumetofen indicate that it is effective against spider mites at 100–800 g a.i./ha with sufficient persistence on fruit trees, tea, and ornamentals. No phytotoxicity has been observed on various varieties of crops at the rate of at least twice as usual field use rates. Because of these characteristics cyflumetofen will be the growers' acaricide of choice as it can be deployed across all crop growth stages with full confidence in the both efficacy and selectivity.

### Introduction
Cyflumetofen was discovered in our studies of acaricidal benzoylacetonitrile compounds and the 20% SC formulation is currently under world-wide development starting in Japan. This paper reports the basic properties and the field performance of cyflumetofen.

### Chemical, physical toxicological properties

ISO name: Cyflumetofen

Structural formula:




Chemical name (IUPAC): 2-methoxyethyl (*RS*)-2-(4-*tert*-butylphenyl)-2-cyano-3-oxo-3-(α,α,α-trifluoro-*o*-tolyl)propionate

CAS RN: *[400882-07-7]*

| | |
|---|---|
| Empirical formula: | $C_{24}H_{24}F_3NO_4$ |
| Molecular weight: | 447.5 |
| Appearance: | white powder |
| Vapour pressure: | $< 5.9 \times 10^{-6}$ Pa at 25°C |
| Solubility in water: | 0.0281 mg/L at 20°C |

**Mammalian toxicity (technical grade)**

| | | |
|---|---|---|
| Acute oral $LD_{50}$, rat (females): | | > 2000 mg/kg body weight |
| Acute dermal $LD_{50}$ (24h), rat (males, females): | | > 5000 mg/kg body weight |
| Acute inhalation $LC_{50}$ (4h), rat (males, females): | | > 2.65 mg/L air |
| Eye irritation, rabbit: | | non-irritant |
| Skin irritation (4h), rabbit: | | non-irritant |
| Skin sensitization, guinea pig: | | positive |
| Mutagenicity: | Ames test | negative |
| | Chromosomal aberration | negative |
| | Micronucleus test | negative |
| Teratogenicity, rat, rabbit: | | negative |
| Carcinogenicity, rat, mouse: | | negative |
| Reproduction, rat: | | no effect |

**Environmental safety (technical grade)**

| | | |
|---|---|---|
| Rainbow trout *Oncorhynchus mykiss*, $LC_{50}$ (96h) | | > 0.63 mg/L |
| *Daphnia magna*, $EC_{50}$ (48h) | | > 0.063 mg/L |
| Algae *Selenastrum capricornutum*, EbC50(0–72h) | | > 0.037 mg/L |
| Northern bobwhite *Colinus virginianus*, | $LD_{50}$ | > 2000 mg/kg |
| | $LC_{50}$ | > 5000 mg/kg diet |
| Earthworm *Eisenia fetida*, $LC_{50}$ | | > 1020 mg/kg soil |

**Environmental fate**

| | |
|---|---|
| $DT_{50}$ in soil | 0.4–1.8 days |
| $DT_{50}$ in water (at 25°C) | 9 days (pH 4) |
| | 5 hours (pH 7) |
| | 12 minutes (pH 9) |

**Biological properties**

**Activity spectrum**

The activity spectrum of cyflumetofen against various pests is summarised in Table 1. Cyflumetofen is a highly selective acaricide. It is effective against *Tetranychus* and *Panonychus* mites, while little active against lepidopteran, homopteran, and thysanopteran pests in laboratory.

Table 1. Activity of cyflumetofen against various pests in laboratory

| | Species | Stage* | $LC_{50}$ (ppm) |
|---|---|---|---|
| Acarina | *Tetranychus urticae* | A | 4.8 |
| | *Tetranychus kanzawai* | A | 1.1 |
| | *Panonychus citri* | A | 0.8 |
| | *Panonychus ulmi* | A | 1.4 |
| Lepidoptera | *Spodoptera litura* | L2 | > 200 |
| | *Plutella xylostella* | L2 | > 200 |
| Homoptera | *Myzus persicae* | L1 | > 200 |
| | *Nephotettix cincticeps* | L2 | > 200 |
| Thysanoptera | *Frankliniella occidentalis* | L2 | > 200 |

*A: adult; L1,L2: 1st and 2nd instar larva

**Activity on developmental stage**

Cyflumetofen is active against all developmental stages of mites, but more so against larvae than adults (Table 2). The $LC_{50}$ values against *T. urticae* adults and larvae were 4.8 and 0.9 ppm, respectively. The $LC_{50}$ values against all developmental stages of *T. kanzawai* and *P. citri* are also < 5 ppm.

Table 2. Activity of cyflumetofen on developmental stages of mites

| Developmental stage | $LC_{50}$ (ppm) | | |
|---|---|---|---|
| | *T. urticae* | *T. kanzawai* | *P.citri* |
| Eggs (incl. dying after hatch) | 2.5 | 3.8 | 2.5 |
| Larvae | 0.9 | 1.7 | 0.8 |
| Nymphochrysalis | 0.8 | 1.4 | 1.0 |
| Protonymphs | 1.0 | 2.1 | 0.9 |
| Deutochrysalis | 2.0 | 2.4 | 1.4 |
| Deutonymphs | 1.9 | 2.8 | 2.4 |
| Teleiochrysalis | 2.4 | 3.3 | 1.5 |
| Female adults | 4.8 | 2.4 | 2.3 |

**Mode of action**

*T. urticae* adults treated with cyflumetofen begin to lose motor coordination about four hours after treatment. Treated adults are paralysed completely within 24 hours. No negative symptoms similar to those of other acaricides have been observed. Cyflumetofen is also partially effective against eggs. Any mites that subsequently hatch, die shortly afterwards.

The biochemical mode of action of cyflumetofen is still unclear. Some data suggest one metabolite of cyflumetofen blocks the mitochondrial complex II in *T. urticae*. Further experiments are necessary to confirm it.

**Cross-resistance studies**

No cross-resistance has been observed between cyflumetofen and other acaricides (Table 3).

Cyflumetofen is effective against several wild *T. urticae* strains which show resistance to existing acaricides. 80 or more wild strains of *T. urticae*, *T. kanzawai*, *P. citri*, and *P. ulmi* which have different susceptibility spectra to existing acaricides show almost same susceptibility to cyflumetofen. No acaricide has been found in which susceptibility is correlated with that of cyflumetofen.

Table 3. Activity of cyflumetofen against susceptible and resistant strains of *T. urticae*

| Strain | resistant to | $LC_{50}$ (ppm) |
|---|---|---|
| Susceptible | - | 4.8 |
| Yamagata A | Fluacrypyrim | 2.2 |
| Yamagata B | METI | 2.4 |
| Nara A | Chlorfenapyr | 5.9 |

**Effects on beneficial arthropods**

Laboratory tests indicate cyflumetofen is safe to a wide variety of beneficial arthropods (Table 4). In field tests, cyflumetofen did not show any adverse effects to *Apis mellifera* or *Amblyseius californicus*.

Table 4. Beneficial arthropods not affected by cyflumetofen at 200 ppm

| | | | |
|---|---|---|---|
| Predators | | | |
| Acarina | *Amblyseius californicus* | Hymenoptera | *Aphidius colemani* |
| | *Amblyseius cucumeris* | | *Aphidius rhopalosiphi* |
| | *Amblyseius womersleyi* | | *Encarsia formosa* |
| | *Phytoseiulus persimilis* | | *Neochrysocharis formosa* |
| | *Typhlodromus pyri* | | *Trichogramma* sp. |
| Coleoptera | *Harmonia axyridis* | Thysanoptera | *Scolothrips takahasii* |
| | *Oligota kashmirica* | Heteroptera | *Orius strigicollis* |
| | *Stethorus japonicus* | Neuroptera | *Chrysoperla carnea* |
| Other beneficial insects | | | |
| Lepidoptera | *Bombyx mori* | Hymenoptera | *Apis mellifera* |
| | | | *Bombus terrestris* |

**Field studies**

Field studies of cyflumetofen 20% SC have been performed on fruit trees, vegetables, tea, and ornamentals. Several results are shown in Tables 5–11. The results indicate cyflumetofen is effective against spider mites at 100–800 g a.i./ha. No phytotoxicity has been observed in all trials at the rate of at least twice as usual field use rates.

Table 5. Control of *Tetranychus urticae* on apple (Nagano, Japan, 2004)

| | Dosage | Number of nymphs and adults per 10 leaves | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | (g a.i./ha) | 0 | 4 | 7 | 14 | 21 | 31 | (DAT) |
| Cyflumetofen 20%SC | 800 | 7.0 | 2.0 | 0.5 | 4.5 | 8.5 | 11.5 | |
| Acequinocyl 15%SC | 600 | 5.0 | 0.0 | 3.0 | 2.0 | 21.0 | 78.5 | |
| Bifenazate 20%SC | 800 | 11.7 | 1.0 | 2.5 | 1.0 | 7.5 | 26.0 | |
| Fluacrypyrim 30%SC | 600 | 3.5 | 0.5 | 2.0 | 4.5 | 43.0 | 32.5 | |
| Control | | 3.5 | 13.0 | 19.0 | 69.0 | 272.5 | 170.5 | |

DAT: Days After Treatment.

Table 6. Control of *Panonychus ulmi* on apple (Nagano, Japan, 2004)

| | Dosage | Number of nymphs and adults per 10 leaves | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | (g a.i./ha) | 0 | 4 | 7 | 14 | 21 | 28 | (DAT) |
| Cyflumetofen 20%SC | 800 | 2.3 | 0.0 | 0.3 | 0.3 | 0.0 | 0.8 | |
| Acequinocyl 15%SC | 600 | 6.8 | 0.3 | 2.0 | 7.8 | 20.8 | 9.0 | |
| Bifenazate 20%SC | 800 | 1.0 | 1.0 | 3.5 | 11.0 | 26.5 | 16.5 | |
| Control | | 2.0 | 7.0 | 20.0 | 29.0 | 150.0 | 25.0 | |

Table 7. Control of *Panonychus citri* on citrus (Shizuoka, Japan, 2001)

| | Dosage | Number of female adults per 10 leaves | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | (g a.i./ha) | 0 | 4 | 7 | 14 | 21 | 28 | (DAT) |
| Cyflumetofen 20%SC | 800 | 2.8 | 0.0 | 0.0 | 0.1 | 1.0 | 0.7 | |
| | 400 | 3.1 | 0.0 | 0.0 | 0.2 | 1.3 | 0.9 | |
| Bifenazate 20%SC | 800 | 3.2 | 0.0 | 0.0 | 0.4 | 3.3 | 10.0 | |
| Control | | 6.0 | 1.5 | 6.6 | 105.8 | 228.3 | 18.4 | |

Table 8. Control of *Brevipalpus phoenicis* on citrus (Santa Fé do Sul, Brazil, 2002)

| | Dosage | % Efficacy | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | (g a.i./ha) | 6 | 14 | 45 | 75 | 105 | 135 | (DAT) |
| Cyflumetofen 20%SC | 100 | 83 | 95 | 100 | 100 | 85 | 82 | |
| | 200 | 81 | 93 | 100 | 100 | 94 | 90 | |
| Cyhexatin 50%SC | 250 | 70 | 83 | 99 | 98 | 83 | 80 | |
| Hexythiazox 50%WP | 30 | 60 | 89 | 86 | 85 | 82 | 77 | |

Table 9. Control of *Tetranychus kanzawai* on pear (Naruto, Japan, 2003)

| | Dosage | Number of female adults per 10 leaves | | | | | |
|---|---|---|---|---|---|---|---|
| | (g a.i./ha) | 0 | 5 | 10 | 20 | 30 | (DAT) |
| Cyflumetofen 20%SC | 800 | 3.0 | 0.0 | 0.0 | 0.1 | 0.8 | |
| Bifenazate 20%SC | 800 | 2.8 | 0.0 | 0.0 | 0.8 | 3.8 | |
| Control | | 2.4 | 7.8 | 10.6 | 17.1 | 45.6 | |

Table 10. Control of *Tetranychus kanzawai* on tea (Kagoshima, Japan, 2002)

| | Dosage | Number of nymphs and adults per leaf | | | | |
|---|---|---|---|---|---|---|
| | (g a.i./ha) | 0 | 8 | 14 | 21 | (DAT) |
| Cyflumetofen 20%SC | 800 | 13.3 | 0.0 | 0.2 | 0.7 | |
| Bifenazate 20%SC | 800 | 25.5 | 8.9 | 15.4 | 7.4 | |
| Milbemectin 1%EC | 40 | 14.6 | 12.4 | 14.3 | 21.1 | |
| Control | | 11.8 | 26.0 | 51.8 | 39.3 | |

Table 11. Control of *Tetranychus urticae* on rose (Elst, Netherlands, 2006)

| | Dosage | Number of adults per 10 leaves | | | | |
|---|---|---|---|---|---|---|
| | (g a.i./ha) | 0/A1 | 7/A1 | 7/A2 | 14/A2 | (DAT) |
| Cyflumetofen 20%SC | 200 | 33 | 8 | 8 | 2 | |
| Bifenazate 24%SC | 96 | 45 | 5 | 9 | 3 | |

*Test materials were applied twice with seven day interval (A1, A2).

**Conclusion**

Cyflumetofen has excellent efficacy and long persistence against spider mites. The efficacy is not affected by developmental stages, resistance to existing acaricides, or any other field conditions. It is suitable for IPM because of its significant safety to predatory arthropods and pollinators. It will be the farmers' acaricide of choice as it can be deployed across all crop growth stages with full confidence in efficacy, crop safety, and selectivity.

## Chlorantraniliprole: a novel anthranilic diamide insecticide

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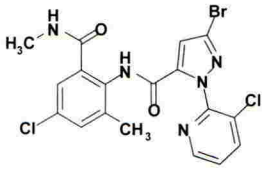
**Abstract**
Chlorantraniliprole (ISO) is a novel insecticide discovered by DuPont, also known as Rynaxypyr and DPX-E2Y45, which belongs to a new chemical class of selective ryanodine receptor (RyR) agonists. Upon ingestion, chlorantraniliprole activates the release and depletion of internal calcium stores in muscles. The insect rapidly stops feeding, becomes paralyzed, and ultimately dies. Differential selectivity towards insect RyRs explains chlorantraniliprole's outstanding profile of mammalian toxicity. It is primarily active on chewing pests by ingestion and secondarily by contact, showing good ovi-larvicidal and larvicidal activity. In some species, efficacy on adults is also observed. Inhibition of insect feeding occurs rapidly (minutes to a few hours after ingestion) and death normally occurs within 24-72 hours. Product development in Europe is currently focused on foliar applications in top fruit and vegetable crops, grapes and potatoes. Rates of 10-60 g a.i./ha are highly effective on many important pests in Europe, such as; *Cydia pomonella*, *Phyllonorychter* spp., *Leucoptera malifoliella*, *Argyrotaenia pulchellana*, *Pandemis* spp., *Adoxophyes orana*, *Cydia molesta*, *Anarsia lineatella*, *Lobesia botrana*, *Eupoecilia ambiguella*, *Leptinotarsa decemlineata*, *Phyllocnistis citrella*, *Spodoptera littoralis*, *S. exigua*, *Helicoverpa armigera*, *Autographa gamma* and *Ostrinia nubilalis*.

Consistency of performance, breadth of spectrum and exceptional crop safety are some of the key product features. Whereas the new mode of action makes chlorantraniliprole a valuable option for IRM strategies, safety to key beneficial arthropods and honeybees confer a strong fit within IPM programmes. The remarkably favorable toxicity profile of chlorantraniliprole, combined with low use rates, provides large margins of safety for consumers and agricultural workers.

**Introduction**
Chlorantraniliprole is being developed worldwide by DuPont in a broad range of crops to control a range of pests belonging to the Order Lepidoptera and some Coleoptera, Diptera

and Isoptera species, at rates between 10-110 g a.i./ha. It possesses a new mode of action (group 28 in the IRAC MoA classification scheme), high biological activity (even against some tough-to-control pests), very low mammalian toxicity and selectivity to non-target arthropods. This paper summarizes the main product features and the results obtained in the experimental work carried out in Europe since 2002.

**Chemical and physical properties**

| | |
|---|---|
| CAS number: | *[500008-45-7]* |
| Common name: | Chlorantraniliprole |
| Chemical class: | Anthranilic diamide |
| Code number: | DPX-E2Y45 |
| Chemical name (CAS): | 3-bromo-*N*-[4-chloro-2-methyl-6-[(methylamino) carbonyl] phenyl]-1-(3-chloro-2-pyridinyl)-1*H*-pyrazole-5-carboxamide |
| Molecular formula: | $C_{18}H_{14}BrCl_2N_5O_2$ |
| Structural formula: |  |

| | |
|---|---|
| Molecular weight: | 483.15 g/mole |
| Physical state (pure a.s.): | Fine crystalline off-white powder |
| Melting point (pure a.s.): | 208-210°C [(200-202°C (technical)] |
| Vapour pressure (20° C): | $6.3 \times 10^{-12}$ Pa |
| Henry's Law Constant (20° C): | $3.2 \times 10^{-9}$ Pa·m$^3$/mole |
| Part. Coeff. oct/w (Log $P_{ow}$) (20° C): | 2.76 (pH 7) |
| Dissociation Constant ($pK_a$) (20° C): | 10.88 |
| Solubility in water (20° C): | 1.0 mg/L |
| Solubility in various solvents (20° C): | acetone - 3446 mg/L , methanol - 1714 mg/L, ethyl acetate - 1144 mg/L |

**Formulations** – Chlorantraniliprole is primarily formulated as a 20% w/v suspension concentrate (Coragen) and as a 35% water dispersible granule (Altacor), both showing good tank-stability and compatibility with conventional crop protection products.

**Mammalian toxicity** – Chlorantraniliprole has low acute oral and dermal toxicity, is not classified as irritating to eyes or skin, and is not a skin sensitizer. It has shown no potential for mutagenicity, developmental effect or carcinogenicity. The chronic toxicity is very low.

| | |
|---|---|
| Acute oral $LD_{50}$ (rat) : | >5000 mg/kg |
| Acute dermal $LD_{50}$ (rat) : | >5000 mg/kg |
| Acute inhalation $LC_{50}$ (4h) (rat): | >5.1 mg/L |
| Dermal irritation (rabbit): | Not irritating |
| Skin sensitization (guinea pig, mouse, LLNA): | Not a sensitizer |
| Mutagenicity (Ames): | Negative |

**Toxicity to wildlife** – Chlorantraniliprole has low acute, dietary and chronic toxicity to

birds and fish. It shows some toxicity to aquatic invertebrates, such as *Daphnia*, but has low toxicity to algae and other aquatic plants, such as *Lemna*.

**Avian**

| | |
|---|---|
| Bird (Bobwhite quail) $LD_{50}$: | >2250 mg/kg |
| Bird (Bobwhite quail) $LC_{50}$: | >5620 ppm in diet [>1729 mg/kg b.w.] |
| Bird (Mallard duck) $LC_{50}$: | >5620 ppm in diet [>2431 mg/kg b.w.] |

**Aquatic**

| | |
|---|---|
| Fish (Rainbow trout) $LC_{50}$: | >13.8 mg/L (solubility limit) |
| Fish (Bluegill sunfish) $LC_{50}$: | >15.7 mg/L (solubility limit) |
| Invertebrate (*Daphnia magna*) $EC_{50}$: | 0.0116 mg/L |
| Algae (*Selenastrum capricornutum*) $EC_{50}$: | >2 mg/L (solubility limit) |
| Duckweed (*Lemna gibba*) $EC_{50}$: | >2 mg/L (solubility limit) |

**Beneficial organisms** – Chlorantraniliprole has an excellent profile with regard to safety to beneficial arthropods and non-target organisms such as earthworms and bees and is highly compatible for use within Integrated Pest Management (IPM) programmes. The effect of chlorantraniliprole on honeybees has been studied extensively (acute oral and laboratory studies and semi-field tunnel tests with *Phacelia* and wheat) demonstrating low intrinsic toxicity and high levels of safety to honey bees under field conditions. The lack of harmful effects on beneficial insects has been demonstrated in laboratory and field studies on a number of key parasitoid and predator species belonging to the Order Hymenoptera (*Braconidae, Aphidiidae, Trichogramatidae, Aphelinidae*), Coleoptera (*Coccinellidae*), Neuroptera (*Chrysopidae*), Heteroptera (*Anthocoridae, Nabidae, Lygaeidae*), Diptera (*Syrphidae*) and Acari (*Phytoseiidae*). This extensive data set on non-target arthropods has demonstrated that chlorantraniliprole does not impact the natural populations under practical use conditions.

| | |
|---|---|
| Earthworm acute $LC_{50}$: | >1000 mg/kg |
| Earthworm reproduction NOEC: | 1000 mg/kg |
| Honeybee acute $LD_{50}$: | >104.1 µg/bee oral |
| | >4 µg/bee contact (sol. limit) |
| Wasp parasitoid (*Aphidius rhopalosiphi*) $LR_{50}$: | >750 g/ha |
| Predatory mite (*Typhlodromus pyri*) $LR_{50}$: | >750 g/ha |

In addition to regulatory studies on indicator species, several field tests have confirmed minimal to no impact upon beneficial arthropods.

Table 1. Safety to *Anthocoris nemoralis* on pear after 2 applns at max. use rate (Italy, 2006) (Means in a column followed by the same letter do not differ significantly at P= 0.05)

| | | | | Assessment seven days after 2nd application | | |
|---|---|---|---|---|---|---|
| | g a.i. / hL | g a.i. / ha | # Apps | Larvae | Adults | Mobile forms |
| DPX-E2Y45 20SC | 3.5 | 52.5 | 1 | 4.56 a,A | 4.31 a,A | 8.88 a,A |
| DPX-E2Y45 20SC | 3.5 | 52.5 | 2 | 5.25 a,A | 4.13 a,A | 9.38 a,A |
| Reference S Pyrethroid | Label Rate | | 2 | 0 b,B | 0.31 b,B | 0.31 b,B |
| Untreated | n.a. | n.a. | n.a. | 5.69 a,A | 4.38 a,A | 10.06 a,A |

**Environmental fate** – Chlorantraniliprole degrades in the environment with a half-life from < two to 12 months in standard studies. Half-lives are shorter in the presence of crop cover. Chlorantraniliprole can be sequestered in soil; and therefore, has limited mobility in the soil. The primary degradation pathway is chemical degradation, with a single major degradation product, which is biologically inactive and does not leach. Chlorantraniliprole degradation rate in soil is not affected by pH.

**Biological properties**

**Biochemical mode of action** – Chlorantraniliprole has a novel mode of action and novel structural type that is not currently found in other commercially available insecticides in Europe. The primary route of entry into target insects is ingestion, with secondary entry via absorption through the cuticle. The biological activity of chlorantraniliprole is due to its ability to activate insect ryanodine receptors (RyRs) (Cordova *et al*, 2006). This activation stimulates release and depletion of calcium from the internal stores of smooth and striated muscle, causing impaired muscle regulation, paralysis and ultimately insect death. Chlorantraniliprole shows excellent differential selectivity toward insect RyRs over mammalian receptors. Inhibition of insect feeding occurs rapidly, from a few minutes to several hours after ingestion, depending on species susceptibility. Treated larvae initially show signs of lethargy and lack of coordination that may be severe enough to cause the larvae to fall from the plant. Insect death normally occurs within 24 to 72 hours of initial ingestion/absorption.

**Spectrum of activity and potency** – Chlorantraniliprole is highly effective on several pests in the Order Lepidoptera and some Coleoptera, Diptera and Isoptera. In Europe, chlorantraniliprole is being developed primarily for foliar applications on fruit and vegetable crops, grapes and potatoes. At 10-60 g a.i./ha, the product has demonstrated effective control of the pest species: *Cydia pomonella*, *Phyllonorychter* spp., *Leucoptera malifoliella*, *Argyrotaenia pulchellana*, *Pandemis* spp., *Adoxophyes orana*, *Cydia molesta*, *Anarsia lineatella*, *Lobesia botrana*, *Eupoecilia ambiguella*, *Leptinotarsa decemlineata*, *Phyllocnistis citrella*, *Spodoptera littoralis*, *S. exigua*, *Helicoverpa armigera*, *Autographa gamma* and *Ostrinia nubilalis*. Chlorantraniliprole has high insecticidal potency with larvae of sensitive species having the laboratory $LC_{50}/LC_{90}$ values that are normally one order of magnitude lower than standard OP (organo-phosphate) and IGR (insect growth regulator) references. For example, the $LC_{50}$ and $LC_{90}$ of chlorantraniliprole against *C. pomonella* neonate larvae is tenfold lower than those of azinphos-methyl or methoxyfenozide. In addition to the potent larvicidal activity, chlorantraniliprole features some true ovicidal activity (a percent of the exposed eggs do not eclose) and substantial ovi-larvicidal activity (neonates emerging from the eggs exposed to the product - either pre or post-oviposition - die during or immediately after eclosion). In some species (*e.g. L. decemlineata*), efficacy against adults has also been demonstrated.

**Anti-resistance strategy** – Chlorantraniliprole possesses a new mode of action (group 28 in the IRAC MoA classification scheme). Although chlorantraniliprole has no cross-resistance with other insecticides, the risk of resistance development has been considered from the beginning. Pro-active, anti-resistance management is an essential part of the marketing strategy of chlorantraniliprole. The product will be recommended for use with a restricted number of applications per season, within spray programmes that include other effective insecticides with different modes of action.

**Field evaluation**

**Methodology** – Field experiments were conducted following EPPO (European & Mediterranean Plant Protection Organization) or local guidelines, in accordance with GEP (Good Experimental Practice). Treatment effects are reported as % reduction over the untreated control using Abbott's formula. The tabulated data represent the mean performance of all the assessments carried out season-long, from all significant trials.

**Pome fruit** – Chlorantraniliprole has demonstrated outstanding codling moth control. Results from sequential applications at 14-day intervals, at the rate of 3.5 g a.i./hl indicate similar to or mostly better performance than the best OP or IGR (MAC) references. Laboratory bioassays (unreported data) show chlorantraniliprole activity on resistant codling moth populations and no cases of cross-resistance are thus far known.

Table 2. Efficacy on codling moth (Europe, 2003-2006)

| Treatment | Dose rate g a.i./hl | % Damage Reduction All fruit | immature fruits | mature fruits | fallen fruits |
|---|---|---|---|---|---|
| DPX-E2Y45 20SC | 2.5 | 82 | 80 | 85 | 82 |
| DPX-E2Y45 20SC | 3.5 | 90 | 89 | 93 | 89 |
| DPX-E2Y45 20SC | 4 | 93 | 92 | 93 | 92 |
| Reference OP | Label Rate | 85 | 87 | 80 | 87 |
| Reference MAC | Label Rate | 75 | 75 | 75 | 74 |

Best results were obtained when chlorantraniliprole was applied before egg-hatch during the embryonic stage of *C. pomonella.* Although the product does not show strong ovicidal activity, the ovicidal timing (from beginning of egg-laying to black-head stage) provides the best overall performance against codling moth. This is explained by the biological availability of the molecule, partial ovicidal effects and potent "ovi-larvicidal effects".

Table 3. Efficacy on 1st generation codling moth at different appln timings (Italy, 2007)

| Treatment | No. of Apps | Appln timing | Dose rate g a.i./ha | Total Fruit Damage |
|---|---|---|---|---|
| DPX-E2Y45 20SC | 1 | T1 egg-laying | 4 | 7.9 |
| DPX-E2Y45 20SC | 1 | T2 black-head | 4 | 8.1 |
| DPX-E2Y45 20SC | 1 | T3 egg-hatch | 4 | 17.8 |
| DPX-E2Y45 20SC fb Ref, OP | 2 | T1 & T1+14 | 4 & label rate | 5.1 |
| DPX-E2Y45 20SC | 2 | T1 & T1+14 | 4 | 4.1 |
| DPX-E2Y45 20SC fb Ref, Oxadiazine | 2 | T1 & T1+14 | 4 & label rate | 4.8 |
| Ref. IGR fb Ref. OP | 2 | T1 & T1+14 | label rates | 7.3 |
| Untreated check | n.a. | n.a. | n.a. | 29.9 |

fb = followed by

Chlorantraniliprole also controls leafminer and leaf roller species (unreported data). Appropriate treatment sequences provide excellent broad-spectrum Lepidoptera control without additional applications.

**Stone fruit** – The sensitivity of *C. molesta* (oriental fruit moth) to chlorantraniliprole in laboratory bioassays was demonstrated to fall within the same range as that of *C. pomonella.* Field tests in peaches and nectarines with repeat applications at 10-12 day intervals showed very good fruit and standard shoot protection, from a rate of 4 g a.i./hl. For control of *A. lineatella* (peach twig borer), a stone fruit borer of higher concern in the Mediterranean area, chlorantraniliprole consistently provided excellent fruit protection, at a rate of 3.5 g a.i./hl.

Table 4. Efficacy on *C. molesta* and *A. lineatella* (Southern Europe, 2004-2006)

| Treatment | Dose rate g a.i./hl | % Reduction fruit damage | | % Reduction shoot damage | |
|---|---|---|---|---|---|
| | | *C. molesta* | *A. lineatella* | *C. molesta* | *A. lineatella* |
| DPX-E2Y45 20SC | 2.5 | 74 | 77 | 66 | 62 |
| DPX-E2Y45 20SC | 3.5 | 75 | 85 | 71 | 83 |
| DPX-E2Y45 20SC | 4 | 85 | 91 | 77 | 83 |
| Reference OP | Label Rate | 80 | 67 | 74 | 60 |
| Ref. Neonicotinoid | Label Rate | 76 | 65 | 74 | 84 |

**Sweet Pepper** – Applied at a 7-10 day interval, chlorantraniliprole effectively controlled all the key Lepidoptera pests of sweet pepper, at a rate between 30 and 40 g a.i./ha (field crops) or 3.5 to 4 g a.i./hl (greenhouse crops). Against *S. exigua* and *O. nubilalis*, chlorantraniliprole showed superior performance over commercial standards, whereas control of *S. littoralis* was comparable to the best standard from the rate of 3.5 g a.i./hl. For caterpillar control in vegetable crops, the best timing for chlorantraniliprole application is when the first significant adult flights occur or the very first damage symptoms are visible on plants.

Table 5. Efficacy on greenhouse pepper (Southern Europe, 2003-2006)

| Treatment | Dose rate g a.i./hl | % Damage reduction Leaf | | Fruit | % Reduction No. larvae | | |
|---|---|---|---|---|---|---|---|
| | | *S. littoralis* | *S. exigua* | *O. nubilalis* | *S. littoralis* | *S. exigua* | *O. nubilalis* |
| DPX-E2Y45 35WG | 2.5 | 54 | 78 | 91 | 95 | 92 | 86 |
| DPX-E2Y45 35WG | 3.5 | 71 | 86 | 96 | 95 | 94 | 90 |
| DPX-E2Y45 35WG | 4 | 79 | 91 | 98 | 100 | 90 | 100 |
| Ref. Oxadiazine | Label Rate | 78 | 72 | 55 | 100 | 75 | 77 |
| Ref. Carbamate | Label Rate | n.a. | n.a. | n.a. | n.a. | n.a. | 60 |
| Ref. Microbial Metabolite | Label Rate | 78 | 71 | 59 | 100 | 80 | n.a. |

**Grapes** – Chlorantraniliprole was extensively tested on wine and table grape crops in Southern and Central Europe on grape berry moth. Results from 3 g a.i./ha and above indicate comparable or higher reduction of fruit damage and larval presence over commercial standards, when the product was applied before egg-hatch (egg-laying to black-head).

Table 6. Efficacy on 2nd generation *Lobesia botrana* (Europe 2003-2006)

| | | % Reduction | | |
|---|---|---|---|---|
| Treatment | g a.i./hl | damaged bunches | damaged berries | larvae |
| DPX-E2Y45 20SC | 2.5 | 73 | 86 | 87 |
| DPX-E2Y45 20SC | 3 | 78 | 90 | 92 |
| DPX-E2Y45 20SC | 3.5 | 80 | 89 | 96 |
| Reference Oxadiazine | Label Rate | 69 | 81 | 88 |
| Reference MAC | Label Rate | 61 | 85 | 91 |

Tomatoes & Eggplants - On field tomatoes, chlorantraniliprole at a rate of 25 to 35 g a.i./ha provided excellent *H. armigera* control and fruit protection, equivalent to or mostly higher than all reference products to which it was compared. For control of the Lepidoptera complex on tomatoes in Southern Europe, the appropriate rate range was from 30 to 40 g a.i./ha (field crops) or from 3.5 to 4 g a.i./hl (greenhouse crops).

Table 7. Efficacy on field tomatoes & eggplants (Southern Europe 2004-2006)

| | | % Damage reduction | | | % Reduction | |
|---|---|---|---|---|---|---|
| | Dose rate | Fruit | | leaf | No. larvae | |
| Treatment | g a.i./ha | *H. armigera* | *S. littoralis* | *S. exigua* | *H.armigera* | *S. littoralis* |
| DPX-E2Y45 35WG | 25 | 81 | 74 | 78 | 88 | 71 |
| DPX-E2Y45 35WG | 35 | 83 | 76 | 86 | 90 | 74 |
| DPX-E2Y45 35WG | 40 | 87 | 83 | 90 | 99 | 80 |
| Ref. Oxadiazine | Label Rate | 70 | 84 | 82 | 89 | 85 |
| Ref. Carbamate | Label Rate | 60 | n.a. | 61 | 85 | 83 |
| Ref. S. Pyrethroid | Label Rate | 63 | n.a. | n.a. | 79 | n.a. |
| Ref. Microbial Metabolite | Label Rate | 53 | 88 | n.a. | 88 | 93 |

**Potatoes** – On potatoes, low rates of chlorantraniliprole (10-12 g a.i./ha) provided excellent control of larvae and adults of *L. decemlineata* for up to 21 days after a foliar application.

Table 8. Efficacy on *L. decemlineata* 1-22 days after 1 application (Europe 2003-2006)

| Treatment | Dose rate, g a.i./ha | % Reduction foliar damage | % Reduction larvae |
|---|---|---|---|
| DPX-E2Y45 20SC | 7.5 | 83 | 92 |
| DPX-E2Y45 20SC | 10 | 89 | 92 |
| DPX-E2Y45 20SC | 12.5 | 91 | 97 |
| Reference S Pyrethroid | Label Rate | 86 | 88 |
| Reference Neonicotinoid | Label Rate | 91 | 95 |

**Lettuce** – In lettuce, results indicate excellent field performance of chlorantraniliprole was equivalent to the best *S. littoralis* reference products.

Table 9. Efficacy on lettuce (Southern Europe 2003-2006)

| Treatment | Dose rate g a.i./ha | % Reduction leaf damage | | % Reduction No. larvae | | |
|---|---|---|---|---|---|---|
| | | *S. littoralis* | *S. exigua* | *S. littoralis* | *S. exigua* | *H. armigera* |
| DPX-E2Y45 35WG | 25 | 74 | 88 | 53 | 94 | 97 |
| DPX-E2Y45 35WG | 35 | 83 | 83 | 87 | 100 | 99 |
| DPX-E2Y45 35WG | 40 | 90 | 92 | 91 | 100 | 99 |
| Ref. Oxadiazine | LR | 82 | 80 | 93 | 93 | 93 |
| Ref. Carbamate | LR | n.a. | 86 | n.a. | 95 | 88 |
| Ref. Microbial Metabolite | LR | 72 | n.a. | 93 | n.a. | n.a. |

LR = Label Rate

## Conclusions

Chlorantraniliprole offers many benefits to agricultural producers in top fruit, vegetables, grapes, potatoes and other crops. In many cases, it represents a new standard in efficacy as well as providing extremely good residual control. It has low recommended use rates (10-60 g a.i./ha in Europe) that, in combination with very low mammalian toxicity, provide a very low risk profile in terms of occupational and dietary exposure.

Chlorantraniliprole provides a new mode of action that is extremely useful in IRM programmes and has minimal impact on beneficial arthropods important for commercial IPM programmes. Overall, this extremely positive profile may be useful in the reduction of risk across a wide range of areas of concern associated with pesticide use, and will provide an invaluable new tool to meet grower needs.

## Acknowledgements

Sincere thanks to all cooperators who contributed to the development of chlorantraniliprole.

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# Spirotetramat, an innovative fully systemic insecticide for sucking insect pest control in agriculture: biological profile and field performance

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## Abstract
The tetramic acid derivative spirotetramat (internal code BYI08330), has shown an outstanding performance against sucking insect pests in laboratory and greenhouse assays as well as in field trials. The product acts – mainly after ingestion – as an inhibitor of lipid biosynthesis affecting juvenile stages with additional effects on adult fecundity. Due to the new mode of action for scales (soft and armoured), mealy bugs, psyllids, whiteflies and aphids, populations resistant to conventional insecticides, are well controlled. Its full two-way systemicity (phloem and xylem mobility) ensures the control of hidden and soil dwelling sucking pests after foliar application, as well as the protection of new shoots.

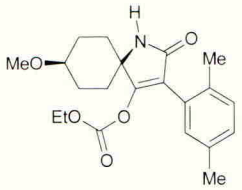
The worldwide field development of spirotetramat in Bayer CropScience AG resulted in numerous uses against many species of whiteflies, aphids, scales, mealybugs, psyllids and selected thrips species in vegetables, cotton, soybean, pome and stone fruit, grapes, hop, citrus, nut trees and banana. It constitutes an excellent rotation partner with existing products for the management of resistant aphids, whiteflies and psyllids. Only moderate effects have been found on beneficial arthropods, which make the product suitable for modern IPM systems.

## Introduction
Sucking pests (i.e. whiteflies, aphids, psyllids, soft and armoured scales) are serious damaging pests in agriculture. However, the number of available chemicals belonging to different chemical classes for their control is relatively limited and development of resistance hampers the use of currently used products. In this context, the development of spirotetramat by Bayer CropScience AG under the brand name Movento® opens new horizons for the control of these pests.

Spirotetramat belongs to the chemical class of tetramic acids and acts as a lipid biosynthesis inhibitor (LBI) (Nauen *et al.*, 2006). Due to its mode of action, juvenile stages of sucking pests are particularly affected. In addition, spirotetramat benefits from unique translocation properties; after penetrating the leaves the product is transformed to spirotetramat-enole, transported within the xylem and the phloem, thereby even providing control of hidden pests (such as root aphids) and the protection of new shoots appearing after foliar application.

In this paper, we present the physicochemical characteristics along with the toxicological and environmental behaviour of the active substance spirotetramat. Furthermore, its biological performance in laboratory, greenhouse and field trials is highlighted.

## Chemical and physical properties

| | |
|---|---|
| Common name (ISO): | spirotetramat |
| Chemical name (IUPAC): | ethyl *cis*-3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl carbonate |
| CAS number: | *[203313-25-1]* |
| Empirical formula: | $C_{21}H_{27}NO_5$ |
| Structural formula: |  |
| Molecular weight: | 373.45 g/mol |
| Colour/appearance: | light beige powder |
| Vapour pressure: | $1.5 \times 10^{-8}$ Pa at 25 °C |
| Water solubility: | 29,9 mg/L at pH 7 |
| Melting point: | 142 °C |

## Human safety

| | |
|---|---|
| Acute oral $LD_{50}$ rat: | > 2000 mg a.i./kg |
| Acute dermal $LC_{50}$ (24h) rat: | > 2000 mg a.i./kg |
| Acute inhalation ($LC_{50}$) rat: | > 4381 mg a.i/$m^3$ |
| Skin irritation (4h) rabbit: | non-irritant |
| Eye irritation (4h) rabbit: | non-irritant |
| Skin sensitization guinea pig: | skin sensitizer |
| Genotoxicity: | no evidence of genotoxic or mutagenic potential |
| Developmental toxicity: | |
| Rat- maternal and developmental NOAEL: | 140 mg/kg bw/day |
| Rabbit- maternal NOAEL: developmental NOAEL: | 10 mg/kg bw/day 40 mg/kg bw/day |
| The studies did not reveal a specific teratogenic potential of spirotetramat | |
| Chronic toxicity: | NOAEL: 13.2 mg/kg bw/day |

## Environmental safety

| | |
|---|---|
| Birds, acute toxicity $LD_{50}$: | > 2000 mg a.i./kg bw |
| Earth worms (*Eisenia fetida*): | > 1000 mg a.i./kg of dry weight soil |
| *Daphnia magna*, $EC_{50}$ (48h): | > 42.7 mg a.i./L |
| $LD_{50}$ (48h, contact), honeybee: | >100 µg a.i./bee |
| $LD_{50}$ (48h, oral), honeybee: | 107.3 µg a.i./bee |
| Predatory mites: (*Typhlodromus pyri,* $LR_{50}$ in lab test) | 0.333 g a.i./ha |
| Micro-hymenoptera: (*Aphidius rhopalosiphi,* $LR_{50}$ in lab test) | 114.7 g a.i./ha |

## Environmental fate

| | |
|---|---|
| Soil degradation, $DT_{50}$: | < 1 d (parent) ; 5 to 23 d (metabolites). |
| Partition coefficient (log P): | 2.73 |

Mobility in soil: no concern with regard to groundwater contamination (parent and metabolites)

| | |
|---|---|
| Microbial mineralization: | no negative effect |
| Aquatic systems: | |
| $DT_{50}$ aerobic aquatic | < 1 day |
| $DT_{50}$ anaerobic aquatic and | |
| $DT_{50}$ aqueous photolysis: | *ca* 3 days |

**Formulations**

Spirotetramat is mainly effective after ingestion. That means that the active ingredient must first penetrate the plant via the leaves. Penetration after foliar spray is enhanced by the addition of adjuvants. Such systems have been developed for example as an Oil Dispersion (OD) containing 150 g a.i./L or as Suspension Concentrates (SCs) containing 48 to 150 g/L. A 240 g a.i./L SC has to be tank-mixed with a suitable adjuvant (such as rapeseed oil methyl ester) in order to exploit the biological efficacy fully. A suspension concentrate containing 120 g/L of spirotetramat and 360 g/L of imidacloprid is also being developed in Brazil for use against aphids, whiteflies, thrips and bugs in cotton, vegetables and soybean. All formulations have demonstrated a good miscibility with conventional crop protection products.

**Translocation properties and biological profile**

Phloem mobility was demonstrated in a test system where the compound was applied only to the first true leaf of cabbage and the biological efficacy was monitored on the third true leaf infested with *Myzus persicae*. High biological activity and a clear dose-response were detected in the third leaf, indicating translocation in the phloem (Table 1). Translocation is especially effective from mature leaves to new growing leaves. Xylem mobile compounds, e.g. neonicotinoids, usually exhibit no activity in this test. The compound has also excellent translaminar efficacy.

Table 1. Phloem-systemic efficacy of spirotetramat (plus 2 g/L rapeseed oil methyl ester) against *M. persicae* on cabbage. Mean values from 9 tests. Mixed populations, efficacy at 7d. Abbreviation: SD = Standard Deviation.

| Rate (µg a.i./1st leaf) | % Efficacy (3rd leaf) | SD |
|---|---|---|
| 100 | 100 | 0 |
| 20 | 100 | 0 |
| 4 | 85.6 | 24 |
| 0.8 | 56.7 | 29.4 |
| 0.16 | 22.5 | 21.9 |
| 0.032 | 8.8 | 14.8 |

Efficacy of spirotetramat is much stronger against immature stages than against adult insects (aphids, whiteflies, psyllids, scales). As shown in Table 2, the $LC_{95}$ for adults is 30 times higher than the $LC_{95}$ for 1st instar nymphs of *M. persicae*. It could be demonstrated in other tests, however, that the fecundity of adult aphids was strongly affected.

Table 2. Efficacy of spirotetramat (+ 1 g/L rapeseed oil methyl ester) against juvenile and adult forms of *Myzus persicae* in a leaf dip bio-assay

| LC (ppm) 6 DAT | 1st instar nymphs | Adults |
|---|---|---|
| $LC_{50}$ | 0.2 | 1.4 |
| $LC_{95}$ | 0.7 | 22.1 |

The speed of lethal effect is variable, depending on the life stages or on external parameters. Usually, after having ingested spirotetramat, juvenile stages of target insects cannot moult properly and die within 2 to 5 days.

**Cross resistance studies**

Resistance studies were conducted on insects which have developed strong resistance to insecticides, such as *Myzus persicae* and *Bemisia tabaci*. For *B. tabaci*, leaf-dip bioassays were done on cotton plants using 2nd instar whitefly nymphs, following the methodology described by Elbert & Nauen (1996) (Table 3). All strains of *B. tabaci*, including Q-type which is resistant to a wide range of insecticides (OP, neonicotinoids), turned out to be susceptible to spirotetramat, with a maximum variation of 3.8 fold between strains. For *M. persicae*, leaf-dip bioassays were done on cabbage using 3rd instar nymphs, according to Nauen & Elbert (2003) (Table 4). Spirotetramat showed excellent efficacy against highly resistant R3 strains.

Spirotetramat will be listed as a member within group 23 of the IRAC (Insecticide Resistance Action Committee) mode of action classification scheme.

Table 3. Log-dose probit – mortality data of spirotetramat on some strains of *B. tabaci*. Leaf-dip bioassay, 2nd instar nymphs, 10d.

| Strain | Biotype | Known Resistance | $LC_{50}$ ppm | RR |
|---|---|---|---|---|
| SUD-S | | Susceptible | 0.26 | |
| CRE04-01 | Q | Multi | 1 | 3.8 |
| ESP-00 | Q | Multi | 0.74 | 2.8 |
| BR-JM03 | B | OP; SP | 0.64 | 2.5 |
| ISR-02 | B | Multi | 0.72 | 2.8 |
| MEX03-02 | B | OP; SP | 0.49 | 1.9 |

Abbreviations: RR = Resistance Ratio; Multi = resistant to OP = organophosphates; neonicotinoids; SP=pyrethroids; buprofezin; pyriproxyfen; pymetrozin.

Table 4. Log-dose probit-mortality data of spirotetramat on some strains of *M. persicae*. Leaf-dip bioassays, 3rd instar nymphs, 3d.

| Strain | Biotype | Known resistance | $LC_{50}$ ppm | RR |
|---|---|---|---|---|
| NS | | susceptible | 0.69 | |
| JR | R3 | OPs; CAR; SP | 0.59 | 0.9 |
| F04-01 | R3 | OPs; CAR; SP | 0.27 | 0.4 |

Abbreviations: RR = Resistance ratio; CAR=carbamates; SP = pyrethroids

**Field performance**

The biological efficacy of spirotetramat against sucking insect pest groups was evaluated in field trials in different countries. Product applications were done in line with local practices.

Control of psyllids - Spirotetramat has demonstrated excellent and long lasting efficacy against important psyllid species such as *Psylla piri*, *P. pyrisuga, Paratrioza cockerelli, Diaphorina citri* respectively in pear, tomato and potato, citrus crops. Optimum application timing against *P. piri* is against the second generation, at the beginning of egg hatch, which allows good insect control and prevents damage for at least three weeks after application (Table 5).

Table 5. Mean efficacy (%) of spirotetramat against *Psylla piri* in pear (13 field trials; Italy and France 2004-2006).

| | Application rate g a.i./ha/mch | 3-4 DAA 6 trials | 7-10 DAA 12 trials | 14-17 DAA 13 trials | 21-23 DAA 12 trials | 28-35 DAA 3 trials |
|---|---|---|---|---|---|---|
| Untreated | - | 26 | 32 | 37 | 28 | 48 |
| spirotetramat | 72-75 | 76 | 81 | 96 | 93 | 73 |
| Abamectin | 6,75 | 84 | 77 | 87 | 79 | 56 |

untreated = number of nymphs/shoot. Spray volume: 500 L/1 mch.
DAA = Days After Application; mch = meter canopy height

Control of scales - Spirotetramat has shown good to excellent efficacy against species from different scale families: soft scales (Coccidae); armoured scales (Diaspididae) and mealybugs (Pseudococcidae). Excellent efficacy against *Aonidiella aurantii* and other economically important scale species in citrus was demonstrated (Table 6). Sprays should be made at the crawler stage. In countries such as Spain, a single application was effective to protect the orange fruits from *A. aurantii* infestation until harvest time. The same excellent performances against the Californian red scale were also detected in the countries where it is considered to be a major pest in citrus crops (USA; South Africa; Morocco; Turkey). In pome and stone fruit, good to excellent control of *Quadraspidiotus perniciosus*, *Lepidosaphes ulmi* and *Pseudaulacaspis pentagona* have been observed in trials conducted in Europe and USA.

Table 6. Mean efficacy (%) of spirotetramat against scales in citrus crops at fruit maturity. Spain, 2004-2006.

| | Rate (g a.i./ha/mch) | *A. aurantii* 9 trials/orange | *Aspidiotus nerii* 7 trials/lemon | *Parlatoria pergandii* 3 trials/orange |
|---|---|---|---|---|
| Untreated | | 72 | 57 | 64 |
| spirotetramat | 60 | 95 | - | - |
| | 75 | - | 84 | 86 |
| pyriproxyfen | 50 | 62 | 62 | 88 |

untreated: % of infested fruits. Spray volume = 1000 L/1 mch
Abbreviation: mch = meter canopy height

Spirotetramat has demonstrated outstanding control of mealybugs on grapes in field trials carried out in the EU (Spain, Greece, Portugal), USA, Mexico and South Africa, providing very good long-term protection from insect damage on bunches until harvest period (Table 7).

Table 7. Efficacy (%) of spirotetramat against mealybugs (*Pseudococcus* spp; *Planococcus* spp) on grapes. Mean from 15 trials, 2004-2006

| | Rate (g a.i./ha) | Europe (11 trials) | USA (4 trials) |
|---|---|---|---|
| untreated | - | 55 | 34 |
| spirotetramat | 75 | 92 | - |
| | 72-88 | - | 99 |
| standard | | 70 | 69 |

untreated: % of infested bunches (EU) or nbr of insects/bunch (USA); standard = OP (chlorpyriphos or methidathion) in EU; buprofezin in USA

Control of whiteflies – All whitefly species are effectively controlled by spirotetramat: in vegetables – *Trialeurodes vaporariorum, B. tabaci, Aleyrodes proletella*, and in citrus, *Aleurothrixus floccosus*. In all cases, the first application with spirotetramat must be made at the beginning of the infestation, when whitefly populations are composed of adults, eggs and young nymphs. Plants were protected from honeydew and sooty mould for at least 4 weeks after the last application, in trials carried out in Spain against *B. tabaci* in pepper. In Brazil, a combination of spirotetramat with imidacloprid proved to be highly effective for whitefly control and for whitefly-transmitted viruses, in beans and tomato (Table 8).

Table 8. Mean efficacy (%) of spirotetramat against *B. tabaci* in vegetable crops. Results from 12 trials in Spain and Brazil, 2004-2006.

| | | 8 trials (Spain) | 4 trials (Brazil) | 3 trials (Brazil) |
|---|---|---|---|---|
| | Rate | Damage control | Insect control | Virus control |
| Untreated | | 68 | 209 | 73 |
| Spirotetramat | 48* | - | 96 | 54 |
| Spirotetramat | 75** | 92 | - | - |
| Spirotetramat+imidacloprid | 24+72* | - | 93 | 78 |
| Imidacloprid | 72* | - | 71 | 58 |
| Imidacloprid | 150** | 47 | - | - |
| Pyriproxyfen | 100* | - | 73 | 27 |

* g a.i./ha. ** g a.i./ha/m leafwall. Untreated: % of sooty mould on leaves and fruits (Spain); number of nymphs/10 $cm^2$ leaf or % of virus-infected plants (Brazil).

Control of aphids – Spirotetramat has provided a very good level of control of a wide range of aphid species, including those which are usually protected from the insecticide spray by their habitat (such as aphid species living on the roots, or hiding in the leaves) or by their secretions (such as the woolly apple aphid). In lettuce, for example, the control of *Nasonovia ribisnigri*, *Aulacorthum solani* and also of the root-aphid *Pemphigus bursarius* reached a very high level (Table 9). In apple, spirotetramat is highly effective against

important species such as *Dysaphis plantaginea*, *Eriosoma lanigerum* and *Aphis pomi*; as a consistent biological efficacy requests a penetration of the compound into the leaves, it is preferable to apply the treatment after flowering when sufficient foliage is present.

In some field studies targeted against insects, mite populations were also present; there are side-effects against tetranychid mites, such as *Panonychus ulmi, Panonychus citri* and *Tetranychus urticae.*

Table 9. Mean efficacy (%) of spirotetramat against aphids on lettuce (field trials in France, Italy, Germany, Belgium, 2004-2006)

| | Rate (g a.i./ha) | *Nasonovia ribis-nigri* Aphids number 12-14 DAA | *Pemphigus bursarius* Colonies number 20-21 DAB |
|---|---|---|---|
| | | 8 trials | 2 trials |
| Untreated | - | 41 | 1.3 |
| Spirotetramat | 75 | 99 | 83 |
| imidacloprid | 100 | 75 | 62 |

DAA = Days after Application; DAB = days after second application.
untreated: number of aphids/plant or colonies/roots .

**Safety to natural enemies**

Selectivity towards beneficial insects and predatory mites is a requirement for a modern IPM-compatible product. Side effects of spirotetramat on beneficial arthropods have been tested in various semi-field and field trials. The field trials were conducted in top fruit (apple, pear), citrus, vine, vegetables (eggplant, tomato, broad bean), rice and cotton. Representative species from most important beneficial arthropod groups like predators (bugs, beetles, hoverflies, lacewings, earwigs, mites) and parasitoids (wasps) were chosen for the trials.

Predatory bugs are antagonists of aphids, thrips, white flies, psyllids. From trials carried out in various crops including vegetables (tomatoes, eggplant), pear and cotton, spirotetramat can be considered as harmless to slightly harmful to the predatory bugs *Anthocoris nemoralis, Macrolophus caliginosus* and *Orius* spp. Larvae of hoverflies (*Episyrphus* spp.) feeding on various pest like aphids, were not harmed by spirotetramat in a semi-field test. The side effects on beneficial spiders which have a wide range of prey were tested in rice, vegetables and cotton. Spirotetramat was classified as harmless to slightly harmful to spiders such as *Lycosa* spp. and *Tetragnatha* spp. The same applies to lacewings (*Chrysopa* spp) in vegetables and cotton, and earwigs (*Forficula auricularia, Doru luteipes)* in cotton and vine. Ladybird beetles are important predators of, for example, aphids. In the semi-field and field trials carried out in vegetables, cotton and citrus, spirotetramat could be classified as harmless to moderately harmful. Species included were *Coccinella* spp., *Stethorus* spp. and *Chilocorus nigritus*. Spirotetramat is safe to hymenopteran parasitoids represented by *Aphelinus mali* (trials conducted in apple), *Aphytis lingnanensis*, *Coccidoxenoides* and *Trichogramma cryptophlebiae* (trials conducted in citrus).

Spirotetramat showed side effects on predatory mites. The level of effect found differed considerably from site to site. While field trials in pome fruit in Germany and Belgium

showed no or only slight effects on *Typhlodromus pyri*, spirotetramat was slightly toxic to toxic for this mite species in trials conducted in northern Italy. In the latter case however, the predatory mite populations recovered within the season and prey/predator-ratio was never affected. In trials conducted in vineyards (Italy, Germany), the effects on *T. pyri* and *Kampimodromus aberrans* could be classified as harmless to slightly harmful.

In conclusion, according to the results obtained from semi-field and field trials, spirotetramat can be considered to be safe to most beneficial insects. There were no long lasting, adverse effects on beneficial bugs, lacewings or parasitoids, and there was never disruption of the balance between the mites and the predatory mites. This good selectivity opens possibilities for the combined use of this product with beneficials. Spirotetramat can, therefore, be recommended for the use in Integrated Pest Management systems.

**Conclusions**

Spirotetramat is a new IPM suitable insecticide against sucking pests whose spectrum of efficacy and intrinsic properties ensure that it will become a highly valuable tool in crop protection. Applied to the foliage, it penetrates through the leaf cuticle and is translocated as spirotetramat-enole via the xylem and the phloem, up to growing shoots and down to the roots. These properties, as well as a good selectivity to natural enemies, and the reduction of the insect pests' fecundity, contribute to its excellent longevity.

The product belongs to the group of tetramic acids derivatives with a new mode of action on a broad range of sucking pests and has no cross-resistance to any other commercially available insecticide. Spirotetramat will be an excellent resistance management tool for sucking pest control.

**Acknowledgements**

Thanks to all colleagues who contributed to the worldwide development of spirotetramat. We also acknowledge the contributions of all departments involved in other areas of development: Discovery, Environmental Safety, Environmental Fate, Ecotoxicology and Formulation Development.

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## Spinetoram (XDE-175): a new spinosyn

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### Chemical structure

Spinetoram is a mixture of two main components, 3′-*O*-ethyl-5,6-dihydro spinosyn J (primary factor) and 3′-*O*-ethyl-spinosyn L (minor factor). As with all spinosyns, the central structure is made of a unique 12-membered macrocyclic ring system that is part of an unusual tetracycle (Sparks *et al.*, 1998). Attached to this complex tetracycle are two sugars; an amino sugar (forosamine) and a neutral sugar which has been synthetically modified, 3′-*O*-ethyl-2′,4′-di-*O*-methyl-rhamnose (Crouse *et al.*, 2007). This novel mixture of molecules exhibits both oral and contact insect activity and is structurally outside the scope of any other class of insecticidal chemistry.

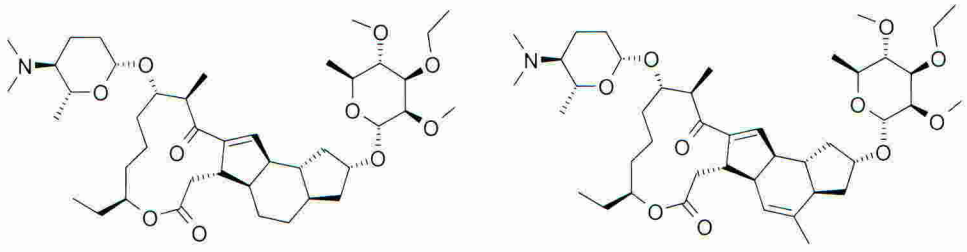

Figure 1. Structure of spinetoram, a mixture comprised of 3′-*O*-ethyl-5,6-dihydro spinosyn J (left) and 3′-*O*-ethyl spinosyn L (right).

**3′-*O*-ethyl-5,6-dihydro spinosyn J (CAS RN: *[187166-40-1]*):**
(2*R*,3a*R*,5a*R*,5b*S*,9*S*,13*S*,14*R*, 16a*S*,16b*R*)-2-(6-deoxy-3-*O*-ethyl-2,4-di-*O*-methyl-α-L-mannopyranosyloxy)-13-[(2*R*,5*S*,6*R*)-5-(dimethylamino)tetrahydro-6-methylpyran-2-yloxy]-9-ethyl-2,3,3a,4,5,5a,5b,6,9,10,11,12,13,14,16a,16b-hexadecahydro-14-methyl-1*H*-*as*-indaceno[3,2-*d*]oxacyclododecine-7,15-dione. (IUPAC)

**3′-*O*-ethyl spinosyn L (CAS RN: *[187166-15-0]*):**
(2*R*,3a*R*,5a*S*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*S*)-2-(6-deoxy-3-*O*-ethyl-2,4-di-*O*-methyl-α-L-mannopyranosyloxy)-13-[(2*R*,5*S*,6*R*)-5-(dimethylamino)tetra-hydro-6-methylpyran-2-yloxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-4,14-dimethyl-1*H*-*as*-indaceno[3,2-*d*]oxacyclododecine-7,15-dione. (IUPAC)

Spinetoram is a fermentation-derived natural product that has been synthetically modified. It originates from the naturally-occurring mixture of spinosyns J and L, which, like spinosad, differ by the presence of a hydrogen or methyl group at the C-6 position. The mixture of these two spinosyns is then *O*-ethylated at the 3′-position of the rhamnose sugar resulting in an ethyl group at the 3′-position, which has been shown to increase greatly the insecticidal activity (Crouse *et al.*, 2001; Sparks *et al.*, 2000). The 5,6-double bond of the major factor (spinosyn J) is then further reduced to form the 5,6-dihydro derivative. The reduction of this particular double bond is associated with improved residuality in the field (Crouse *et al.*, 2007).

Technical grade spinetoram is an off-white solid with a musty odor. The physical properties of the two primary components that constitute spinetoram are shown in Table 1.

Table 1. Physical properties of the two main components of Spinetoram.

| Property | 3′-*O*-ethyl-5,6-dihydro spinosyn J | 3′-*O*-ethyl spinosyn L |
|---|---|---|
| Empirical formula | $C_{42}$ $H_{69}$ N $O_{10}$ | $C_{43}$ $H_{69}$ N $O_{10}$ |
| Molecular weight | 748.019 | 760.030 |
| Melting point, C | 143.4 | 70.8 |
| Log $P^*$ (pH 5, 20°C) | 2.44 | 2.94 |
| Log $P$ (pH 7, 20°C) | 4.09 | 4.49 |
| Log $P$ (pH 9, 20°C) | 4.22 | 4.82 |
| Water solubility, mg/L (pH 5, 20°C) | 423 | 1630 |
| Water solubility, mg/L (pH 7, 20°C) | 11.3 | 46.7 |
| Water solubility, mg/L (pH 10, 20°C) | 6.27 | 0.706 |
| $pK_a$ (25°C) | 7.86 | 7.59 |
| Vapor pressure, Pa (20°C) | $5.3\times10^{-5}$ | $2.1\times10^{-5}$ |

*$P$ = Octanol-water partitioning coefficient

**Discovery of spinetoram**

Spinetoram is the direct result of a novel approach to compound optimization; specifically, the application of an artificial neural network (ANN) to understand quantitative structure activity relationships (QSAR). The application of ANNs to the problem of spinosyn QSAR identified the 3′-ethyl as critical to improving biological activity (Sparks *et al.*, 2000; Sparks *et al.*, 2006). This modification to the basic spinosyn structure, coupled with hydrogenation of the 5,6-double bond for improved residuality led to the discovery of spinetoram (Sparks *et al.*, 2006). Thus, from its roots in a novel natural products program to the use of artificial intelligence to define the best analogs to make, the origin of spinetoram indeed unique in the field of pesticide chemistry.

**Biological activity**

The two small structural differences between spinetoram and spinosad confer very significant differences in the pest management attributes of the two insecticides (Dripps *et al.*, 2006). Spinetoram is intrinsically more potent than spinosad. When injected into the haemolymph of fourth instar *Spodoptera exigua* larvae at 0.1 µg/larva, spinetoram and spinosad are equal in potency, with 96% of spinetoram-treated larvae and 87% of spinosad-treated larvae showing symptoms at 24 hours. When injected at 0.01 µg/larva, however, spinetoram causes 71% of larvae to show symptoms compared to only 4% of larvae injected with spinosad. Spinetoram is faster-acting and demonstrates greater contact activity than spinosad at equivalent rates of exposure. When second instar *S. exigua* larvae are exposed to glass Petri dishes sprayed with 250 ppm solutions of spinetoram or spinosad, spinetoram causes onset of mortality at one hour compared to at least four hours for spinosad. After 24 hours of exposure, spinetoram causes 90% mortality compared to 30% for spinosad. Spinetoram demonstrates a greater breadth of spectrum than spinosad at equivalent rates of exposure. For example, the $LC_{90}$ values for spinetoram against *Spodoptera exigua*, *Helicoverpa zea*, *Plutella xylostella* and *Cydia pomonella* in a diet are all less than 0.053 ppm, whereas $LC_{90}$ values for spinosad range from 0.2 to 0.58 ppm,

and $LC_{90}$ values for indoxacarb range from 0.08 to 0.67 ppm. Finally, spinetoram provides longer residual control than spinosad. When *Cydia pomonella* larvae are exposed to field-treated apples, spinetoram at 105 g a.i./ha provides at least 70% control for 14 days after application, as does azinphos-methyl at 840 g a.i./ha; spinosad achieves this level of control for only three days.

The insecticidal potency and enhanced residual activity of spinetoram enable it to control a wide spectrum of insect pests. To date, spinetoram has demonstrated activity against pest species within the following orders and families of insects: Lepidoptera (Tortricidae, Noctuidae, Plutellidae, Pyralidae, Gracillaridae, Pieridae, Geometridae, and Zygaenidae), Diptera (Agromyzidae, Tephritidae, Muscidae), Thysanoptera (Thripidae), Homoptera (Psyllidae), Coleoptera (Chrysomelidae, Curculionidae), Siphonaptera (Pulicidae), Isoptera (Rhinotermitidae), and Orthoptera (Tettigoniidae, Gryllotalpidae).

**Mode of action**

The spinosyns, spinetoram and spinosad, do not act via the target sites of avermectins, neonicotinoids, pyrethroids, or any other known insecticide (Crouse *et al.*, 2007). The spinosyns act through a novel site in the nicotinic receptor that is distinct from neonicotinoids or any other nicotinic actives (Crouse *et al.*, 2007). Selection for spinosad resistance in *Drosophila* and subsequent sequencing of the genes involved have identified the spinosyn target as an α7-like nicotinic acetylcholine receptor known as Dmα6-nACHR (Orr *et al.*, 2006). It is the activation of this α6-nACHR by the spinosyns that begins the cascade of events leading to insect death. Since both spinetoram and spinosad share the same mode of action, any possible resistance to spinosad will confer resistance to spinetoram. Thus, spinetoram and spinosad should not be rotated with each other.

**Effects on non-target organisms**

Spinetoram has demonstrated low acute toxicity to mammals (Table 2). There have been no indications of mutagenicity, teratogenicity, or oncogenicity based on negative results in the Ames test, chromosomal aberration test, mutation assay, and mouse bone marrow micronucleus assay.

Spinetoram exhibits low toxicity to other non-target organisms such as birds, fish, and earthworms (Table 2).

Spinetoram is toxic to honeybees when bees come into contact with, or consume fresh residues. However, residues aged three hours or longer are practically non-toxic to honeybees. At three hours after application, no mortality and no behavioral effects were observed among actively foraging bees introduced into large field cages covering alfalfa that had been treated with spinetoram at 110 g a.i./ha.

Spinetoram has little toxicity to predatory insect species such as coccinellids and lacewings, but is toxic to predatory mites and insect parasitoids in acute laboratory tests. Under field conditions, however, any observed effects on beneficial species have been slight and transitory. The short environmental persistence of spinetoram minimizes exposure to beneficial species (Haile *et al.*, 2006).

Table 2. Toxicological properties of Spinetoram.

| Toxicological test | Species | Endpoint |
|---|---|---|
| Acute oral $LD_{50}$ | F344/DuCrl Rat (♂&♀) | >5000 mg/kg bw |
| Acute dermal $LD_{50}$ | F344/DuCrl Rat (♂&♀) | >5000 mg/kg bw |
| Acute inhalation $LC_{50}$ | F344/DuCrl Rat (♂&♀) | >5.5 mg/L |
| Avian acute oral $LD_{50}$ (14 d) | Mallard duck | >2250 mg/kg |
| | Bobwhite quail | >2250 mg/kg |
| Avian acute dietary $LC_{50}$ (8 d) | Mallard duck | >5620 mg/kg diet |
| | Bobwhite quail | >5620 mg/kg diet |
| Avian reproduction NOEC | Mallard duck | 1000 mg/kg diet |
| | Bobwhite quail | 1000 mg/kg diet |
| Fish acute $LC_{50}$ (96 hr) | Rainbow trout | >3.46 mg/L |
| | Bluegill sunfish | 2.69 mg/L |
| Daphnid acute $LC_{50}$ (48 hr) | *Daphnia magna* | >3.17 mg/L |
| Earthworm acute $LC_{50}$ (14 d) | *Eisenia foetida* | >1000 mg/kg soil |

**Environmental fate**

Spinetoram is rapidly biodegraded in soil, the terrestrial field dissipation half-life ranges from three to five days. Spinetoram is also rapidly degraded in natural surface waters; its aquatic field dissipation half-life is less than one day. This rapid degradation in surface waters minimizes the potential for exposure to aquatic non-target organisms.

**Efficacy**

Extensive field evaluation in Europe, the USA (Yoshida *et al.*, 2006; Olson *et al.*, 2006) and other areas in the world has demonstrated the excellent efficacy profile of spinetoram against key pests of pome and stone fruits, vines and tree nuts. At a rate of 36 g a.i./ha spinetoram provided control of 95% of grape berry moth (*Polychrosis [Lobesia] botrana*) in vines and was equivalent to or better than indoxacarb (45-54 g a.i./ha), chlorpyrifos (520-940 g a.i./ha) and flufenoxuron (72-80 g a.i./ha). Against codling moth (*Cydia pomonella*) in apples, spinetoram was very effective at 100 g a.i./ha offering control of 92% and being equivalent to thiacloprid (120-144 g a.i./ha) and superior to azinphos-methyl (500-750 g a.i./ha) and chlorpyrifos (675-975 g a.i./ha). Against oriental fruit moth (*Cydia [Grapholita] molesta*) in peaches, at the rate of 100 g a.i./ha spinetoram provided commercial levels of control equivalent to that achieved by deltamethrin (17.5 g a.i./ha) and much better than those offered by spinosad (216 g a.i./ha) and azinphos-methyl (500-750 g a.i./ha). Finally, against *Psylla* spp. in pears, spinetoram showed an excellent efficacy profile at 100 g a.i./ha (94% control) outperforming abamectin (13.5-18 g a.i./ha), thiacloprid (144-175 g a.i./ha) and phosmet (500 g a.i./ha).

Spinetoram has also shown very good performance against key pests of cotton and vegetables in Northern Africa and Middle East countries as well as vegetable crops in the USA (Richardson *et al.*, 2006; Weiss *et al.*, 2006). The effective rates against these pests are presented in Table 3.

In addition to use for controlling pests of tree fruits, vines, and vegetables, the utility of spinetoram is being explored in other crops such as maize, rice, tea, and oil palm.

Table 3. Effective use rates of Spinetoram for major insect pests of cotton and vegetables.

| Insect pest | Crop | Rate (g a.i./ha) |
|---|---|---|
| *Helicoverpa armigera* | Cotton, vegetables | 24-60 |
| *Spodoptera littoralis* | Cotton, vegetables | 10-60 |
| *Leptinotarsa decemlineata* | Potatoes | 24 |
| *Liriomyza* spp. | Tomatoes | 48-72 |
| *Plutella xylostella* | Cole crops | 44-53 |
| *Trichoplusia ni* | Cole crops | 44-53 |
| *Frankliniella occidentalis* | Fruiting vegetables | 44-70 |
| *Thrips tabaci* | Onions | 50-70 |

**Registration timeline**

Spinetoram was accepted for evaluation by US EPA under its reduced risk pesticide initiative. US federal registration is anticipated in late 2007. Submission for Annex I inclusion in the European Union is planned for late 2007 and EU member state submissions are expected to begin in 2008. Registration and development of spinetoram in many other countries around the world is anticipated.

**Summary**

Spinetoram represents a significant advance in spinosyn pest management technology. It provides greater activity, longer residual control, and a broader spectrum of control than spinosad, while maintaining the very favorable human health and environmental profiles that were pioneered by spinosad a decade ago. Spinetoram is active against a wide range of pest insects, including key pest species among the Lepidoptera, Diptera, Thysanoptera, Coleoptera, Orthoptera, Isoptera, and certain Homoptera. Spinetoram controls key pome and stone fruit pests such as codling moth, *Cydia pomonella*; oriental fruit moth, *Cydia [Grapholita] molesta*; and pear psyllids, *Psylla* spp. In vegetable crops, spinetoram controls *Spodoptera* spp. and most other pest Lepidoptera, and is highly effective against thrips and dipterous leafminers. Spinetoram conserves most beneficial arthropods in vegetables and tree fruits, and will be an effective and compatible control option for integrated pest management (IPM) programs.

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# Metaflumizone, a new broad-spectrum insecticide for crop protection

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## Abstract
Metaflumizone is a new insecticide belonging to the semicarbazone class of chemistry. It has demonstrated utility in controlling a broad range of insect pests in crop and non-crop situations. It provides good to excellent activity on most economically important Lepidoptera species as well as certain important pests in the orders Coleoptera, Hemiptera, Hymenoptera, Diptera, Isoptera and Siphonaptera. It has a favourable toxicological and environmental profile and is considered safe to beneficial insects including pollinators. It is the only sodium channel blocker insecticide that does not require bioactivation and has been classified by IRAC as the sole representative of the mode of action classification Group 22B. The unique profile of metaflumizone, including lack of cross-resistance with conventional insecticides makes it an important tool for use in integrated pest management and insecticide resistance management programmes.

## Introduction
Metaflumizone is a new insecticide belonging to the semicarbazone class of chemistry and was discovered by Nihon Nohyaku Co., Ltd. It is being globally co-developed by BASF and Nihon Nohyaku. BASF is developing the compound for crop and non-crop markets in North America, Europe, Asia and Central & South America. Registrations have recently been granted in Indonesia, Colombia, Austria and Germany with product launches planned in 2007 under the brand names ALVERDE and VERISMO.

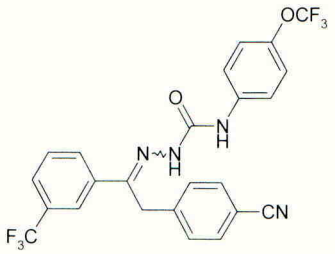
Extensive research and development has demonstrated the utility of metaflumizone in controlling a broad and diverse range of insects across the Orders: Lepidoptera, Coleoptera, Hemiptera, Hymenoptera, Diptera, Isoptera and Siphonaptera in crop and non-crop markets. Key crop market segments include tuberous and corm vegetables, *Brassica* vegetables, leafy vegetables, fruiting vegetables and cotton. Additional crops and uses will continue to be added.

With its favourable environmental and toxicology profile, including low mammalian toxicity, safety to beneficial insects (including insect predators and pollinators), and its novel mode of action, metaflumizone will be an important addition to the modern chemicals available for use in insect control programs. Furthermore, research results demonstrate its utility in controlling resistant insect pests through its novel mode of action as the only member of the Insecticide Resistance Action Committee's (IRAC), mode of action Group 22B and no known cross resistance to other commercial insecticides.

This paper describes the physicochemical and biological profiles of metaflumizone based on extensive laboratory, glasshouse and field trials.

## Chemical and physical properties

Structural formula




| | |
|---|---|
| BSI Common name (ISO): | Metaflumizone |
| Code Number: | 4080134 |
| CAS Number: | *[139968-49-3]* |
| Chemical Name (IUPAC): | (*E*,*Z*)-2-[2-(4-cyanophenyl)-1-(3-trifluoromethylphenyl) ethylidene]-*N*-(4-(trifluoromethoxyphenyl) hydrazine carboxamide |
| Molecular formula: | $C_{24}H_{16}F_6N_4O_2$ |
| Molecular weight: | 506.40 g/mol |
| Appearance: | White powder at room temperature |
| Odour: | Faint aromatic at room temperature |
| Melting point: | 197 °C (*E*)-isomer and 154 °C (*Z*)-isomer |
| Density: | 1.461 g/cm³ at 20°C |
| Partition coefficient (*n*-octanol/water): | 5.1 (*E*)-isomer and 4.4 (*Z*)-isomer |

## Mammalian toxicology

| | |
|---|---|
| Acute oral $LD_{50}$ (rat) | >2000 mg/kg (males/females) |
| Acute dermal $LD_{50}$ (rat) | >4000 mg/kg (males/females) |
| Acute inhalation $LC_{50}$ (mouse) | > 5.2 mg/L |
| Eye irritation (rabbit) | Non irritating |
| Skin irritation (rabbit) | Non irritating |
| Skin sensitization (guinea pig) | Non sensitizing |
| 2-generation reproduction (rat) | NOEC=20 mg/kg bw/day (270 ppm dietary) |

Metaflumizone is not mutagenic, teratogenic or carcinogenic

## Environmental fate

| | |
|---|---|
| Soil mobility | no leaching problems |
| Outdoor dissipation | ½ life of 4.3 – 27 days |
| Vapor pressure | $1.24 \times 10^{-5}$ Pa at 20 °C (*E*/*Z*)-isomers |
| Water solubility | $1.79 \times 10^{-3}$ mg/L, 20 °C (*E*/*Z*)-isomers |

**Effects on non-target organisms**

| | |
|---|---|
| Rainbow trout (96-hour acute $LC_{50}$, water only) | > 343 ppb |
| Bluegill sunfish (96-hour acute $LC_{50}$, water only) | > 349 ppb |
| Channel catfish (96-hour acute $LC_{50}$, water/sediment exposure) | > 300 ppb (water)<br>> 1 ppm (sediment) |
| Carp (96-hour acute $LC_{50}$, water/sediment exposure) | > 300 ppb (water)<br>> 1 ppm (sediment) |
| Daphnia (48-hour acute $EC_{50}$) | > 331 ppb |
| Green alga (96-hour acute $EC_{50}$) | > 313 ppb |
| Mysid shrimp (96-hour acute $EC_{50}$) | > 289 ppb |
| Mallard duck (acute oral $LD_{50}$) | > 2000 mg a.i./kg bw |
| Northern bobwhite (acute oral $LD_{50}$) | > 2000 mg a.i./kg bw |
| Mallard duck (reproduction study) | NOEC = 7.5 ppm |
| Northern bobwhite (reproduction study) | = 15 ppm |

**Effects on beneficial arthropods**

Metaflumizone has extremely low toxicity to beneficial insects and earthworms.

| | |
|---|---|
| Honeybees, *Apis mellifera*: | |
| 48-hour contact $LD_{50}$ (US EPA protocol)* | > 106 $\mu$g a.i./bee |
| 96-hour contact $LD_{50}$ (EU protocol)** | > 1.65 $\mu$g a.i./bee |
| 96-hour oral $LD_{50}$ (EU protocol) | > 2.43 $\mu$g a.i./bee |
| Cage test (EU protocol) | Acceptable risk |
| * = dorsal exposure; ** = venter exposure) | |
| Earthworm ($LC_{50}$, 14 d) | > 1000 mg a.i./ kg soil |

Extensive studies conducted under laboratory and field conditions have demonstrated low impact of metaflumizone on key beneficial insects such as Pirate bugs (*Orius* spp.), Lacewings (*Chrysopa* spp.), Wasps (*Trichogramma* spp.), Damsel bugs (*Nabis* spp.), Big-eyed bugs (*Geocoris* spp.) and predatory mites (*Amblyseius* spp.).

**Biological profile**

**Spectrum of activity**

The biological profile of metaflumizone includes good to excellent activity against the larval stages of most economically important Lepidoptera species and adults, larvae and nymphs of certain pests of economic importance in the orders Coleoptera (beetles), Hemiptera (bugs), Hymenoptera (ants), Diptera (flies), Isoptera (termites) and Siphonoptera (fleas).

The spectrum list in Table 1 is not intended to be all-inclusive, but rather to illustrate the specific activity of metaflumizone on certain key target pests.

Table 1. Insecticidal spectrum of metaflumizone on key insect pests of agriculture

| Scientific name | Common name | Rates (g a.i./ha) |
|---|---|---|
| **Lepidoptera** | | |
| *Spodoptera littoralis* | Armyworm | 220 – 240 |
| *Spodoptera eridania* | Southern armyworm | 200 - 240 |
| *Spodoptera exigua* | Beet armyworm | 240 |
| *Spodoptera litura* | Cluster caterpillar | 240 |
| *Agrotis ypsilon* | Black cutworm | 220 – 260 |
| *Helicoverpa armigera* | Old world bollworm | 220 - 240 |
| *Helicoverpa assulta* | Bollworm | 200 – 240 |
| *Helicoverpa zea* | Corn earworm | 240 - 288 |
| *Heliothis virescens* | Tobacco budworm | 168 - 240 |
| *Mamestra brassicae* | Cabbage armyworm | 240 |
| *Crocidolomia binotalis* | Cabbage caterpillar | 240 |
| *Trichoplusia ni* | Cabbage looper | 288 |
| *Plusia intermixta* | Cabbage looper | 240 - 288 |
| *Hellula* spp. | Cabbage webworm | 240 |
| *Alabama argilacea* | Cotton leafworm | 240 |
| *Plutella xylostella* | Diamondback moth | 200 - 240 |
| *Pieris rapae* | Imported cabbageworm | 240 - 288 |
| *Diaphania nitidalis* | Pickleworm | 240 |
| *Pectinophora gossipiella* | Pink bollworm | 240 |
| *Phthorimaea operculella* | Potato tuber moth | 240 - 288 |
| *Cnaphalocrocis medinalis* | Rice leafroller | 120 - 240 |
| *Ostrinia nubilalis* | European cornborer | 240 |
| *Earias insulana* | Spiny bollworm | 240 |
| *Manduca sexta* | Tobacco hornworm | 240 |
| *Tuta absoluta* | Tomato leaf miner | 240 |
| *Plusia gamma* | Tomato looper | 240 |
| *Chrysodeixis chalcytes* | Tomato looper | 240 - 288 |
| *Keiferia lycopersicella* | Tomato pinworm | 240 |
| **Coleoptera** | | |
| *Leptinotarsa decemlineata* | Colorado potato beetle | 60 - 80 |
| *Diabrotica* spp. | Corn rootworm (adults) | 240 |
| *Diabrotica speciosa* | Corn rootworm (larvae) | 240 (in furrow) |
| *Epitrix* spp., *Phyllotreta* spp. | Flea beetles | 240 |
| *Conorhynchus mendicus* | Sugar beet weevil | 240 - 288 |
| *Otiorrynchus mendicus* | Vine weevil | 224 |
| **Hemiptera** | | |
| *Pseudatomoscelis seriatus* | Cotton fleahopper | 240 - 288 |
| *Lygus* spp. | Tarnished plant bug | 288 |
| **Hymenoptera** | | |
| *Solenopsis* spp. | Fire ant | 1.1 |

Specific examples of the control performance of metaflumizone are given below.

The Old World bollworm (*Helicoverpa armigera*) is a major pest of cotton and vegetable crops in southern Europe, Africa and Asia. It can be particularly problematic because it has developed resistance to most commercial insecticide classes. In a field trial on sweet pepper in Spain, metaflumizone provided excellent control of *H. armigera* with two applications and exceeded the performance of the standard (Table 2).

Table 2. Activity of metaflumizone against the Old world bollworm (*Helicoverpa armigera*) on sweet pepper. Spain/2004.

| | Rate | No. of larvae of instars L1-5 per plot (7 DAT) | | | | |
|---|---|---|---|---|---|---|
| Treatment | (g ai/ha) | L1 | L2 | L3 | L4 | L5 |
| Untreated control | - | 1.3 | 1.5 | 2.3 | 0.5 | 0.8 |
| Metaflumizone | 180 | 0 | 1.3 | 0.8 | 0 | 0 |
| Metaflumizone | 240 | 0 | 0 | 0 | 0 | 0 |
| Indoxacarb | 37.5 | 0 | 1.3 | 0.8 | 0.5 | 0 |

The Colorado potato beetle (*Leptinotarsa decemlineata*) is one of the major insect pests of potato throughout Europe and North America. The intensive use of insecticides to control this has led to the development of resistant populations to organophosphates, carbamates, pyrethroids and neonicotinoids and most recently reduced susceptibility to spinosad. Metaflumizone is very active against adults and larvae of the Colorado potato beetle at rates of 60 g a.i./ha and can provide residual control beyond 30 days after a single treatment (DAT) (Table 3).

Table 3. Activity of metaflumizone against Colorado potato beetle *(Leptinotarsa decemlineata)* on potato. Poland/2004

| Compound | Rate | % Control | | | | |
|---|---|---|---|---|---|---|
| | g a.i./ha | 3 DAT | 7 DAT | 14 DAT | 21 DAT | 30 DAT |
| Metaflumizone | 48 | 97 | 100 | 100 | 94 | 89 |
| Metaflumizone | 60 | 100 | 100 | 100 | 100 | 100 |
| Thiacloprid | 36 | 98 | 97 | 94 | 90 | 97 |
| Thiamethoxam | 20 | 99 | 100 | 92 | 94 | 96 |
| Deltamethrin | 5 | 98 | 100 | 82 | 58 | 71 |

Among the most important insect pests of tomato and other fruiting vegetables are the looper caterpillars of the genus *Plusia*. They can cause serious damage to fruiting structures thereby reducing fruit quality as well as yield. Metaflumizone at rates of 200-240 g a.i./ha is very active against a large number of Lepidoptera species including *Plusia* spp., (Table 4). Metaflumizone applied at 180 and 240 g a.i./ha provided superior protection of fruit resulting in improved fruit quality.

Table 4. Activity of metaflumizone against tomato looper (*Plusia* spp.) on tomato. Spain/2004

| Treatment | Rate | % Fruit / Class | | | |
|---|---|---|---|---|---|
| | g a.i./ha | 1 | 2 | 3 | 4 |
| Untreated | - | 35 | 6 | 4 | 55 |
| Metaflumizone | 180 | 78.6 | 5.6 | 1.6 | 14.6 |
| Metaflumizone | 240 | 82.6 | 9.6 | 1.6 | 5.6 |
| Indoxacarb | 37.5 | 73.6 | 8.6 | 1 | 17 |

Class 1 = better quality - Class 4 = less quality; 2 applications (7 days spray interval)

**Mode of action**

Metaflumizone represents a new class of chemistry and it functions by directly blocking voltage-dependent sodium channels in insects without requiring a metabolic activation step. On the basis of its primary target site of action it is classified as a voltage dependent sodium channel blocker insecticide, in IRAC Group 22. The only other member of Group 22 is indoxacarb. Group 22 has now been separated by IRAC into two sub-groups based on definitive differences in the metabolic profile and chemical structure between indoxacarb and metaflumizone. On this basis, metaflumizone has been classified by IRAC into Sub-group 22 B Voltage Dependent Sodium Channel Blockers.

The specific site of insecticidal action of metaflumizone is currently unknown, but it does act on the insect nervous system, where it blocks the voltage-dependent sodium neuron channel. As a result, these neurons are inactivated, causing the insect to enter a state of 'relaxed paralysis' where the effects are the cessation of feeding, increasing levels of immobility and ultimately, death.

The relaxed paralysis caused by metaflumizone is distinct from the knockdown typical of many older insecticides. Insects may physically remain on the crop for several days following the ingestion of metaflumizone residues. Their activity, however, becomes increasingly limited. Nonetheless, feeding stops from between 15 minutes to 12 hours after treatment. Insect death occurs from within an hour and up to 72 hours after treatment. The condition that causes the relaxed paralysis of the insect induces cessation of feeding and prevents fruit and crop damage.

**Cross resistance**

Extensive studies with metaflumizone indicate that there is no known cross-resistance to insect strains resistant to carbamate, organophosphate, pyrethroid, benzoylurea, macrocyclic lactone or indoxacarb insecticides. This makes it an ideal candidate insecticide for use in insect resistance management (IRM) where it is necessary to rotate insecticide classes in order to slow the development of insecticide resistance or as a replacement product in a programme where metabolic resistance exists. Additionally, metaflumizone's low impact on insect predators also makes it a natural choice for insect pest management (IPM).

Data for the Diamondback moth (*Plutella xylostella*) and Tobacco budworm (*Heliothis virescens*) provides two examples of the lack of cross resistance to indoxacarb and the synthetic pyrethroids.

The Diamondback moth has an extraordinary propensity to develop resistance to insecticides. Resistance or reduced susceptibility to a wide range of insecticides has been reported in all agricultural areas throughout the world. Metaflumizone at rates of 240-280 g a.i./ha is very active against this pest.

A pyrethroid-resistant strain of Diamondback moth with 19921-fold resistance to cypermethrin was 94-fold resistant to indoxacarb but not significantly resistant (Resistance-Ratio (R-R) < 2x) to metaflumizone (Table 5). This laboratory colony of *P. xylostella* was established from individuals collected from field locations near Baggio, Philippines, where populations of this pest species have been documented to express resistance to multiple classes of insecticide (including pyrethroids, acylureas and organophosphates).

Table 5. Potency against pyrethroid-Resistant and -Susceptible strains of *Plutella xylostella* (3rd-instars; cabbage leaf-dip assay). Philippines/1999

| Compound | $LC_{90}$ (95% CI) in ppm at 5 DAT | | |
|---|---|---|---|
| | S-strain | R-strain | S/R ratio |
| Metaflumizone | 3.21 (1.96 - 4.51) | 5.22 | 1.6× |
| Indoxacarb | 3.72 (3.32 - 4.30) | 25.25 | 94× |
| Cypermethrin | 0.04 (0.03 - 0.06) | 796.84 | 19921× |

Third-instar tobacco budworm exhibiting 730, 2740 and 1000-fold resistance to cypermethrin, cyhalothrin and deltamethrin, respectively, were 11-fold resistant to indoxacarb, but showed no resistance to metaflumizone. The addition of the metabolic inhibitor PBO reduced the resistance ratio (R-R) for indoxacarb from 11× to 4×, indicating that the indoxacarb resistance was probably P450-based. PBO did not inhibit or synergize metaflumizone activity *vs.* the susceptible or resistant strain (Table 6). This laboratory strain of *H. virescens* has been shown to express both metabolic (oxidative and hydrolytic) and target site mechanisms of resistance to pyrethroids.

Table 6. Potency against pyrethroid-Resistant and -Susceptible strains of *Heliothis virescens* (3rd-instars; cotton leaf-dip assay). Princeton, NJ – USA/1998

| | $LC_{90}$ (95% CI) in ppm at 5 DAT | | |
|---|---|---|---|
| | S-strain | R-strain | s/r ratio |
| Metaflumizone | 0.29 (0.25 - 0.34) | 0.40 (0.32 - 0.70) | NS |
| Indoxacarb | 0.27 (0.16 - 1.08) | 3.19 (1.85 - 10.30)* | 11× |
| Indoxacarb + PBO | N.T. | 1.10 (0.96 - 1.70)* | 4× |
| Cypermethrin | 5.24 (4.31 - 6.77) | 3796.00 (2098 - 12139)* | 724× |

PBO concentration was 1000 ppm; NS = non-significant; * = Significant; N.T. = Not tested

Used in rotation with existing registered insecticide products, metaflumizone is an excellent resistance management tool. However, as with any insecticide, a sound resistance monitoring and management plan is necessary to ensure the sustainable use of metaflumizone.

**Crop selectivity**

Metaflumizone has demonstrated excellent crop safety, selectivity, and no phytotoxicity, even at three times the proposed label rates in all crops tested, including broadacre crops, fruits, vegetables, legumes, tuber crops, citrus, grapes, pome and stone fruits and coffee. Seedling emergence, stem growth, foliage development and dry matter accumulation were unaffected by metaflumizone.

**Conclusions**

The broad pest spectrum, favourable toxicological and environmental profile and low impact on insect predators make metaflumizone an important addition to the toolbox of modern novel insecticides for integrated pest management. Further, its unique pharmacology and lack of cross resistance to commercial insecticides make it an ideal candidate insecticide for use in insect resistance management programmes where it is necessary to rotate insecticide classes in order to slow the development of insecticide resistance to the products available to growers.

## Mesotrione to control triazine- and ALS-resistant *Amaranthus* in grain sorghum

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Grain sorghum (*Sorghum bicolor*) is an important crop in the drier areas of the US Great Plains. However, it is considered a 'minor crop,' and weed control options for sorghum are few, compared to maize. Some sorghum weeds, especially certain *Amaranthus* biotypes, are resistant to the triazine and ALS-inhibitor herbicides used in grain sorghum.

Mesotrione is registered for use on maize (*Zea mays*), applied as an early-preplant, pre-emergence, or post-emergence herbicide. Soil-applied mesotrione, in combination with *S*-metolachlor and atrazine, or foliar-applied mesotrione, in combination with atrazine and crop oil concentrate, effectively controls *Amaranthus* species and many other weeds common in maize. Trials conducted by Kansas State University scientists have shown that grain sorghum has adequate tolerance to soil-applied mesotrione in combination with *S*-metolachlor and atrazine. The objective of this research was to compare the efficacy of mesotrione plus *S*-metolachlor plus atrazine to that of conventional pre-emergence and post-emergence herbicide treatments, for control of triazine- and ALS-resistant Palmer amaranth (*A. palmeri*) biotypes in grain sorghum.

### Materials and methods

In 2006, experiments following the same protocol were established at Clearwater and at Colwich, KS, to determine the efficacy of Lumax, a product of Syngenta Crop Protection, containing mesotrione plus *S*-metolachlor plus atrazine, for control of Palmer amaranth thought to be resistant to triazine and ALS-inhibitor herbicides. These proceedings will elaborate on the Colwich site only.

Grain sorghum (variety: Pioneer 85G01) was planted into a tilled seedbed of silt loam soil in rows 76 cm apart, at a rate of 90,000 seed/ha, on 15 June, 2006. Plot size was 2.3 × 7.7 m, and treatments were replicated four times. Herbicide treatments were broadcast applied through Spray Systems 'turbo-tee' spray tips at 140 l/ha spray volume. Pre-emergence treatments were applied on 18 June, and post-emergence treatments were applied on 7 July.

The experimental site was a farm field with a history of Palmer amaranth escaping control. The 'weed-free treatment' was achieved by applying mesotrione plus *S*-metolachlor plus atrazine, followed with hand weeding as needed. Rainfall following planting was 25 mm on 16 June, 12 mm on 19 June, and 39 mm on 22 June. Palmer amaranth pressure was very heavy, with over 2500 seedlings/$m^2$ in the untreated check plots. Almost no other weeds were present. Weed control was rated on a 0-100 scale, where 90 is 'very good to excellent control,' 70 is 'somewhat less than satisfactory control,' and 50 is 'deficient to moderate control.' On 24 August, sorghum plants were clipped at ground level, oven dried and weighed.

Table 1. Herbicidal control of *Amaranthus palmeri* in grain sorghum at Colwich, KS. Pre-emergence and post-emergence treatments were applied 3 and 21 days after planting, respectively.

| | Rate (g/ha) | Percent control (days after planting) | | | Yield (kg/ha) |
|---|---|---|---|---|---|
| | | 21 | 35 | 63 | |
| Pre-emergence treatments | | | | | |
| Mesotrione + *S*-moc + atrazine | 188 + 1879 + 701 | 91 | 65 | 59 | 3787 |
| *S*-moc + atrazine | 1124 + 1452 | 70 | 41 | 15 | 2246 |
| *S*-moc | 1071 | 51 | 13 | 13 | 820 |
| Atrazine | 1571 | 13 | 3 | 3 | 727 |
| Pre-emergence / Post-emergence | | | | | |
| *S*-moc fb bromoxynil | 1071 / 280 | 54 | 19 | 11 | 1482 |
| *S*-moc fb prosulfuron + COC* | 1071 / 53 | 45 | 20 | 14 | 1239 |
| *S*-moc fb dicamba | 1071 / 280 | 36 | 48 | 36 | 3297 |
| *S*-moc fb carfentrazone + NIS* | 1071 / 8 | 58 | 34 | 16 | 2285 |
| *S*-moc fb 2,4-D amine | 1071 / 533 | 45 | 48 | 33 | 3007 |
| Untreated check | - | - | - | - | 390 |
| Weed-free check | - | - | - | - | 5753 |
| $LSD_{(0.05)}$ | | 15 | 21 | 16 | 1215 |

*Crop oil concentrate and non-ionic surfactant, respectively. fb = followed by
*S*-moc = *S*-metolachlor

**Results and discussion**

At 21 days after planting (see Table 1), the three way product containing mesotrione plus *S*-metolachlor plus atrazine gave very good control of Palmer amaranth. From a distance, plots appeared to be weed-free, but closer examination showed that about six Palmer amaranth seedlings per plot were not controlled. With time, these few uncontrolled plants had a significant impact on sorghum production. Control in the 'standard' pre-emergence treatment of *S*-metolachlor plus atrazine was deficient from the start. Pre-emergence atrazine gave the poorest pre-emergence control, strongly suggesting that most of the Palmer amaranth population was triazine resistant.

*S*-metolachlor is a common pre-emergence herbicide for use in grain sorghum, primarily targeting annual grasses and small-seeded broadleaf weeds like the amaranths. The 1071 g/ha rate used alone in the pre-emergence treatment, and ahead of the post-emergence treatments, controlled about 50% of amaranth seedlings.

Of the post-emergence treatments, dicamba and 2,4-D gave the most amaranth suppression. Prosulfuron is an ALS-inhibitor herbicide, and has systemic activity, but its failure to control strongly suggests ALS resistance in the amaranth population. Bromoxynil and carfentrazone, contact herbicides that kill via foliar burn, were not effective on Palmer amaranth plants that were 12-25 cm tall at post-emergence application.

Soil-applied mesotrione plus *S*-metolachlor plus atrazine appears to have excellent potential for weed control in grain sorghum. Preplant and pre-emergence split applications improve control and are compatible with prevalent no-till sorghum production practices.

# New uses for old chemistry – chlorpyrifos-methyl for the control of pyrethroid resistant *Meligethes* (pollen beetle) in oilseed rape

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**Abstract**
With the loss of many active ingredients as a result of the European re-registration process, the range of insecticides available for resistance management has been reduced. There is widespread resistance in *Meligethes aeneus* (pollen or blossom beetle) populations in Northern Europe to pyrethroid insecticides. With little other chemistry available for the control of pyrethroid resistant pollen beetle, chlorpyrifos-methyl (Reldan), is being developed for use in oilseed rape for the control of this pest. Chlopyrifos-methyl is one of the few organophosphate insecticides to obtain an Annex I listing under 91/414.

This insecticide can be used to control pollen beetle populations that are currently resistant to pyrethroids or as a management tool to prevent the build up of pyrethroid resistance in susceptible populations. Rates between 338 – 450 g a.i./ha give ~80% control of the pest beetle. No cross-resistance between chlorpyrifos-methyl and the pyrethroid lambda-cyhalothrin was detected in field collected populations of *M. aeneus*.

As pollen beetle applications are made close to or at flowering, safety to honeybees (*Apis mellifera*) is an important factor. However, cage studies in oilseed rape have shown no effect on honey bees when not sprayed directly onto bees even when applied at full flowering. Residues were not toxic to foraging bees and bee foraging intensity was not affected.

## Introduction

The blossom or pollen beetle (*Meligethes aeneus* - Nitidulidae) is a serious pest of oilseed rape (*Brassica napus*) crops in northern Europe. There has been relatively few insecticidal modes of action developed for controlling this coleopteran pest; pyrethroids have been a key tool, but resistance to this class of chemistry is now widespread in Germany, France, Denmark and Poland (Ballanger *et al* (2003); Hansen (2003); Heimbach *et al* (2006); Wegorek *et al* (2006)). *Meligethes* causes most damage to oilseed rape when it attacks the unopened flower buds, laying eggs in holes chewed in the base of the buds. Later attacks, when the crop is in full flower, are less damaging and pollen beetles may play a minor role pollination of the crop. Chlorpyrifos-methyl (Reldan 225 g/l EC) is an organophosphate insecticide with a relatively short persistence in crops. This attribute is important to its

development as a tool for pollen beetle control by destroying beetles attacking the unopened buds, but having a short residuality to ensure safety to beneficial insects such as bees (*Apis mellifera*) and other pollinators. An evaluation programme was commenced in 2005 to evaluate chlorpyrifos-methyl for the control of *M. aeneus* in oilseed rape, to determine the cross resistance status of the compound to a pyrethroid and to evaluate the safety of the compound to honey bees.

## Materials and methods

### Field efficacy and yield

Field trials were conducted in Germany, France and the UK in the 2005 – 2007 seasons against *M. aeneus*. Plots of oilseed rape (40 – 50 $m^2$, 4 replicates) were sprayed using pressurized plot sprayers equipped with flat fan nozzles. Test materials were chlorpyrifos-methyl, deltamethrin (Decis Protech 15 g/l EW and lambda-cyhalothrin (Karate Zeon 100 g/l CS). One application was made at growth stage BBCH 55-57 and assessments of adult pollen beetles made at various intervals after application on 25 - 50 samples/plot. In 2006, four trials in Germany were taken to yield using small plot combine harvesters.

### Susceptibility of field collected populations of *M. aeneus* to insecticides

*M. aeneus* adults were collected from six locations in Germany. 'Rotilabo' cups were treated with dilutions of the formulated test substances (either chlorpyrifos-methyl or lambda-cyhalothrin), 10 beetles were placed in each cup and kept at 18-20°C until assessment 5 hours after treatment.

### Cage studies with bees (*Apis mellifera*)

Polythene tunnels (40$m^2$) were erected over flowering oilseed rape plots; three replications were carried out for each treatment. Active bee hives were placed in each polythene tunnel and allowed to acclimatise for three days before insecticide applications were made. Chlorpyrifos-methyl and control (water) applications were made in the early evening when bees were not actively foraging; the toxic standard (dimethoate) was applied directly to foraging bees. Assessments were made of dead bees and bee foraging both before and after application.

## Results

### Field efficacy and yield

Chlorpyrifos-methyl at 338 and 450 g a.i./ha gave >80% control of adult pollen beetles at 1-2 days after application (DAA); the pyrethroid insecticides lambda-cyhalothrin and deltamethrin gave <60% control of pollen beetle in the same time period. Pollen beetle attacks in oilseed rape usually continue into the crop flowering season; efficacy of all treatments declined with time. Chlorpyrifos-methyl treatments maintained >70% control at 3-5 DAA and >65% control at 6-10 DAA (Table 1).

Table 1. Efficacy of chlorpyrifos-methyl and pyrethroid insecticides against adult pollen beetles (*Meligethes aeneus*) in oilseed rape
(20 trials. Germany, France, UK – 2005 - 2007)

| Treatment (g a.i./ha) | | % Control 1 – 2 DAA | % Control 3 – 5 DAA | % Control 6 – 10 DAA |
|---|---|---|---|---|
| chlorpyrifos-methyl | 225 | 76.5 | 64.7 | 62.2 |
| chlorpyrifos-methyl | 338 | 80.2 | 72.1 | 66.4 |
| chlorpyrifos-methyl | 450 | 84.3 | 77.1 | 67.9 |
| lambda-cyhalothrin | 5 | 57.6 | 52.6 | 43.9 |
| deltamethrin | 5 | 58.7 | 52.8 | 44.9 |

Four trials in Germany were taken to yield in 2006; chlorpyrifos-methyl at 338 and 450 g a.i./ha gave yield increases of 9 and 12% respectively. Deltamethrin and lambda-cyhalothrin showed yield increases of 3% (Table 2).

Table 2. Yield increases of oilseed rape after treatment with chlorpyriphos-methyl or pyrethroid insecticides (four trials, Germany 2006)

| Treatment | Rate (g a.i./ha) | % Yield relative to control |
|---|---|---|
| chlorpyrifos-methyl | 338 | 109.64 a |
| chlorpyrifos-methyl | 450 | 112.01 a |
| lambda-cyhalothrin | 5 | 103.16 b |
| deltamethrin | 5 | 102.52 b |
| untreated (tonnes/ha) | | (2.80) c |

Means followed by the same letter do not differ significantly. (P = 0.5 Student Neuman-Keuls)

**Susceptibility of field collected populations of *M. aeneus* to insecticides**

The calculated $LD_{50}$s for lambda-cyhalothrin varied between 0.035 and 7.238 ppm and for chlorpyrifos-methyl between 10.288 and 17.785 ppm. As true susceptibility baselines were not available, relative Resistance Factors (RF) were calculated from the $LD_{50}$s of the population showing the greatest susceptibility to the pyrethroid or organophosphate insecticide. In the populations sampled in 2006, the relative RF values for lambda-cyhalothrin varied between 28.29 and 206.80 and for chlorpyrifos-methyl between 1.02 and 1.73. No evidence of cross-resistance was seen (Table 3). Additional populations are being sampled and evaluated in 2007.

Table 3. Calculated $LD_{50}$ (ppm) and relative resistance factors (1) of lambda-cyhalothrin and chlorpyrifos-methyl against *Meligethes aeneus* adults collected from various sites in Germany in May or June 2006.

| | lambda-cyhalothrin | | chlorpyrifos-methyl | |
|---|---|---|---|---|
| | $LD_{50}$ (5 hours) | Relative RF | $LD_{50}$ (5 hours) | Relative RF |
| Keindorf - S-A | 0.990 | 28.29 | 15.590 | 1.52 |
| Ahlum – NI | 0.035 | - | 10.483 | 1.02 |
| Offenstetten – BAY | 7.238 | 206.8 | 17.785 | 1.73 |
| Welschbillig - R-P | 1.056 | 30.17 | 10.288 | - |
| Regensberg – BAY | 5.409 | 154.54 | 12.922 | 1.26 |

(1) Relative Resistance Factor (RF) calculated from population with the lowest $LD_{50}$

**Cage studies with bees (*Apis mellifera*)**

Bees were assessed for three days before treatment and for seven days post treatment. Chlorpyrifos-methyl at 338 and 450 g a.i./ha showed no significant effects on honeybee mortality post-treatment of the oilseed rape crop (Table 4). The toxic standard, dimethoate, applied at the relatively low rate of 260 g a.i./ha directly to bees, resulted in high levels of mortality immediately post-application and for several days post-application.

Table 4. Effects of chlorpyrifos-methyl on honeybee mortality (tunnel test in oilseed rape)

| Time (days) | Mean number of dead bees | | | |
|---|---|---|---|---|
| | Chp-me 338 g a.i./ha | Chp-me 450 g a.i./ha | Control | Dimethoate 260 g a.i./ha |
| -3 | 13.0 | 7.0 | 15.7 | 13.7 |
| -2 | 10.0 | 8.5 | 12.0 | 15.3 |
| -1 | 29.7 | 37.0 | 14.3 | 19.7 |
| 0 | 18.0 | 28.5* | 4.0 | 224.7* |
| 1 | 14.3 | 9.5 | 6.0 | 45.0* |
| 2 | 9.0 | 9.5 | 6.3 | 119.0* |
| 3 | 9.0 | 9.5 | 9.3 | 37.7 |
| 4 | 4.3 | 4.0 | 5.0 | 23.3 |
| 5 | 5.3 | 3.0 | 5.3 | 27.3 |
| 6 | 4.7 | 2.5 | 4.3 | 26.0 |
| 7 | 6.0 | 1.0 | 10.3 | 20.3 |

ANOVA – GLM, Log n+1 transformed data. *Denotes statistically significant difference from the Control. Dunnett's one-sided test (>Control)

Chlorpyrifos-methyl had no effect on honeybee foraging rates post-treatment. Foraging was significantly reduced after treatment with the toxic reference, dimethoate, and reduced foraging was observed for the duration of the seven days of post-treatment assessments (Table 5).

Table 5. Effects of chlorpyrifos-methyl on honeybee foraging (tunnel test in oilseed rape)

| Time (days) | Mean number of dead bees | | | |
|---|---|---|---|---|
| | Chp-me 338 g a.i./ha | Chp-me 450 g a.i./ha | Control | Dimethoate 260 g a.i./ha |
| -3 | 4.7 | 5.0 | 6.3 | 5.3 |
| -2 | 4.7 | 3.5 | 4.0 | 3.3 |
| -1 | 7.3 | 9.0 | 8.0 | 9.0 |
| 0 | 9.7 | 8.3 | 10.9 | 4.4* |
| 1 | 7.3 | 6.0 | 8.3 | 0.0* |
| 2 | 9.7 | 7.5 | 9.7 | 0.0* |
| 3 | 9.0 | 8.5 | 10.3 | 0.0* |
| 4 | 12.0 | 10.0 | 11.3 | 1.3* |
| 5 | 12.7 | 11.0 | 12. 3 | 1.3* |
| 6 | 11.7 | 11.0 | 11.7 | 1.3* |
| 7 | 8.3 | 6.5 | 9.7 | 5.7 |

ANOVA – GLM, Log n+1 transformed data. *Denotes statistically significant difference from the Control. Dunnett's two-sided test (>Control)

The results from mortality and foraging assessments indicate that if chlorpyrifos-methyl is applied to the crop, rather than the foraging bees, it has no deleterious effects at rates which control pollen beetle. This is consistent with a use pattern under, for example, the German B2 classification, when applications are permitted on flowering crops after the daily bee flight has finished. Although chlorpyrifos-methyl is aimed at applications during growth stages BBCH 51-59, some open flowers (BBCH >60) are often present in the crop; any bees foraging to these early flowers will not be harmed by chlorpyrifos-methyl applied according to label specifications

**Conclusions**

Chlorpyrifos-methyl provides excellent control of pollen beetles, *M. aeneus*, when applied as the 225 g/l EC formulation at 338 or 450 g a.i./ha (1.5 or 2.0 litres/Product/ha) to oilseed rape at the critical application timing for this pest (BBCH 51 – 59). Applications were safe to the crop and a positive yield benefit (9 -12%) was observed.

At the rates applied, chlorpyrifos-methyl is safe to foraging bees and can be applied to crops where bees are actively foraging if it is applied in the early evening when bees are not active.

The lack of cross-resistance of this organophosphate insecticide to pyrethroid resistant *M. aeneus* provides a tool for resistance management. It can be used in those countries where resistance is already an issue to control pollen beetles resistant to pyrethroids or in those countries, such as the UK, where pyrethroid resistance is not yet widespread, to manage resistance development in the future.

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## IKR-001: a novel repellent product effective on whiteflies

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**Abstract**
A repellent product, the active ingredient of which is acetylated monoglycerides (ADI: NL, USA: 21CFR172.828, EU:E472(a)), well known and widely used as a general food additive, was invented for adult whitefly control in glasshouses. Adult whiteflies of species *Bemisia tabaci* or *Trialeurodes vaporariorum* approached and landed on treated plants in the same manner as untreated leaves. However, they left the treated leaves after a brief stay, not settling on the host plants. Sufficient efficacy was observed for a 500 fold diluted foliar spray in field trials on protected tomatoes and eggplants. This product, which is named IKR-001, is under development for agrochemical registration by Ishihara Sangyo Kaisha, Ltd. IKR-001 can be considered to be a safer product for human health and the environment because we have been ingesting it for a long time via food products. IKR-001 will be a promising agent for controlling whiteflies within IPM systems.

**Introduction**
*Bemisia tabaci* is an important pest because it can establish on a wide variety of vegetable crops and can cause both direct sucking damage to plants and also transmit destructive plant disease viruses such as Tomato Yellow Leaf Curl Virus (Makkouk, 1978). Generally, whitefly populations readily develop resistance against chemical insecticides (Wool *et al*, 1990; Prabhaker *et al*, 2005). Therefore, it is not sustainable to depend upon specific agrochemicals in pest control systems, especially for some protected crops which have a long cultivation period. This is one of the reasons why Integrated Pest Management using biological control is being accepted by horticultural farmers. Safer pest control agents are required nowadays as part of IPM techniques. It is considered that creating new pesticides from foodstuffs or food additives which have been consumed for a long time is one of the favorable approaches to make new, safer pesticides. Some products have already been launched in this way, for example EC formulation of propylene glycol fatty acid ester is used for controlling spider mites. We found acetylated monoglycerides show good repellent activity for adult whiteflies while optimizing their formulation from a series of screening trials using general food additives or foodstuffs. We finally composed an emulsifiable concentrate product containing 80% acetylated monoglycerides and named it 'IKR-001'. This report describes the biological properties and performance of IKR-001 against whiteflies, mainly *Bemisia tabaci*.

**Biological properties**
In a glasshouse test, IKR-001 at 1ml/L was applied to potted cucumber seedlings.

Then, treated and untreated pots were located between potted cabbage leaves on which there were adequate numbers of adults of *B. tabaci* (B biotype). The repellent activity was assessed by counting the number of adults on both treated and untreated cucumber leaves

one or three days after treatment. The results of three replicated trials showed that only one adult was observed on the treated plot against 57 individuals in the untreated plot in trial one (1 DAT), 15 versus 212 in trial two (3DAT) and 0 versus 30 in trial three (1DAT). Similar results were observed in the case of *B. tabaci* on tomato and eggplant and *T. vaporariorum* on kidney bean and cucumber. The practical dose rate of IKR-001 was determined at 2ml/L from the other series of trials. To clarify the residual activity, a trial was conducted in a glasshouse on potted tomatoes. IKR-001 at 2ml/L was applied and after that, four treated pots and the same numbers of untreated pots were alternately placed in two lines in a glasshouse in which there were several tomato pots as inoculation sources of *B. tabaci*. The second application was made seven days after the first application.

Table 1. Residual activity of IKR-001 to *Bemisia tabaci* on tomato plants

| | Dose | No. of adults/4 plants, DAT | | | |
|---|---|---|---|---|---|
| | | 2 | 7 | 14 | 21 |
| IKR-001 | 2ml/L | 6 (0) | 42 (1) | 72 (0.8) | 656.5 (65) |
| Control (Water) | | 20 (1) | 78 (8) | 256 (50) | 762.3 (75) |

DAT = Days after 1st treatment
Figures in parentheses show the number of pairs of whitefly

The control value of IKR-001 was 70% 2 days after the first application, decreasing to 46% 7 DAT. However, 72% control was shown seven days after the second application. There was no remaining efficacy 14 days after the second application. The residual activity of IKR-001 was almost seven days. IKR-001 has an activity that interrupts pairing of adult whiteflies on the treated leaves. This efficacy was also strongly confirmed in the trial. Because unfertilized female whiteflies generally produce only male offspring, the activity of pairing interruption may operate effectively to control them.

**The mechanism of repellent action**

Both a treated and an untreated cucumber seedling were placed in a plastic case (260×340×340mm) after spraying with IKR-001 at 2ml/L or water (control). As an inoculation source, an excised cucumber leaf with 150 *B. tabaci* adults was carefully placed at the same distance and height from both pots. The number of adults flying onto the upper side of leaves, leaving the leaves, and moving to the underside of the leaves by walking were counted for one hour. The number of adults on the upper/under side were also counted just one hour after the beginning the trial. Three replications were made.

There was no difference in the number of adults landing on the leaf between the treated and untreated plots. Interestingly, 88% of adults moved to the underside in the untreated plot but only 3% in the treated one. In the treated plot, 68% of landing whiteflies fled from the leaf. This result elucidated that the repellent action of IKR-001 occurred after whiteflies landed, but interfered with their orientation flight into host plants. IKR-001 probably inhibits whiteflies from detecting cues about suitable hosts from the treated plant. Further examination will be needed to clarify whether whiteflies rub or tap their labium on the plant surface and insert stylets into leaf tissue or not.

Table 2. The behavior of adult *B. tabaci* on treated/untreated leaf surface in cucumber

| | Mean number of whiteflies/leaf | | | | |
|---|---|---|---|---|---|
| | Landing[(a)] | Leaving[(b)] | Moving[(c)] | Upper side[(d)] | Underside[(e)] |
| IKR-001(2ml/L) | 10.3 | 7.0 | 0.3 | 3.0 | 1.0 |
| Control (Water) | 11.0 | 0.67 | 9.67 | 0.67 | 13.0 |

(a) No. of whiteflies landing on upper side in 1 hour observation
(b) No. of whiteflies leaving the leaf in 1 hour observation
(c) No. of whiteflies walking to underside in 1 hour observation
(d) No. of whiteflies on upper side at the end of observation
(e) No. of whiteflies on underside at the end of observation, including directly flying there

**Insecticidal activity against eggs and larvae**

The efficacy for different stages of whitefly was determined in laboratory trials. It is possible that IKR-001 possesses insecticidal activity against whitefly by covering their spiracles because acetylated monoglycerides are classified as a kind of fat and fatty oil. Egg plant seedlings were placed in a plastic case (260×340×340mm). 500 adult *B. tabaci* were released into the case and allowed to oviposit freely. After 24 to 48 hours of oviposition, egg plant seedlings were separated from the whiteflies. IKR-001 at 2ml/L was applied by leaf dipping at one to two days, 10-11 days, and 15-16 days after the oviposition period. Mortality of larvae with time was assessed by stereomicroscopic examination. Observation of the emergence hole was also made at the final assessment.

Table 3. Efficacy of IKR-001 at 2ml/L to each stage of *B. tabaci* on egg plant

| Application timing | Mortality, DAT | | | | |
|---|---|---|---|---|---|
| | 8 | 10 | 15 | 17 | 28 |
| 1-2 days after oviposition (Egg) | 13% | 16% | 10% | 11% | 11% |
| 10-11 days after oviposition (Early stage instar) | | | 53% | 56% | 55% |
| 15-16 days after oviposition (mature instar) | | | | 5% | 20% |

DAT = Days after treatment for egg stage

IKR-001 showed 55% mortality to *B. tabaci* only at the early stage instars, but the activity on egg and late stage instars was practically zero. Insecticidal activity was limited, but it might enhance the efficacy of IKR-001 under the actual field conditions.

**Field study**

A field efficacy trial was conducted on protected tomatoes from May to June 2006. Foliar sprays of IKR-001 at 2ml/L were made once or two to three times consecutively with seven day intervals. Applications were started a week after transplanting. A large number of *B. tabaci* were released into the glasshouse at one day and 15 days after transplanting.

There was no clear difference for the control values between one and two applications. The best result in IKR-001 was three applications in which 84% control was shown two weeks after the final application (table.4).

Table 4. Control of adult *Bemisia tabaci* on tomatoes

| | No. of spray | Mean No. of adult *B. tabaci*/plant, DAT | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 4 | 7 | 11 | 14 | 18 | 21 | 28 |
| IKR-001 | 1 | 3 | 2 | 34 | 27 | 16 | 107 | 232 |
| | 2 | 5 | 3 | 36 | 28 | 26 | 35 | 227 |
| | 3 | 5 | 2 | 16 | 11 | 3 | 9 | 66 |
| Acetamiprid | 1 | 0 | 0 | 9 | 7 | 4 | 7 | 31 |
| Control (water*) | 3 | 23 | 13 | 62 | 78 | 42 | 89 | 349 |

DAT = Days after 1st treatment, * water with surfactant

**Conclusion**

IKR-001 is a novel repellent product for controlling adult whiteflies by interrupting settlement and pairing on treated leaves. The actual dose rate is 2ml/L and at this rate, repellent activity lasts almost a week. Moreover, IKR-001 showed moderate insecticidal activity to early stage instars if the spray solution directly contacted them. However, the repellent activity of IKR-001 is not perfect. In laboratory trials, the repellent efficacy was not sufficient unless untreated plants served as preferred hosts. Whiteflies are reluctant to settle on the treated leaves, and they prefer to establish in untreated plants. But if there are no untreated plants in the area they reluctantly establish on treated plants ultimately. Therefore, it is considered that using yellow sticky traps together with IKR-001 is one possible measure to enhance the repellent efficacy securing a "place to go" for whiteflies in actual glasshouses.

The active ingredient, acetylated monoglycerides, has been used and consumed as a plasticizer for chewing gum base for a long time. The other 20% of inert ingredients of this formulation consists of safe substances which are listed on the EPA Inert List 4A, 4B. Thus, we regard the safety of this product as very high. As public concern regarding old pesticide substance safety increases, consumers are demanding that producers minimize the use of conventional pesticides in cultivation. Safer control agents such as IKR-001 will be a useful alternative for IPM in protected horticulture.

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## Coumarin derivatives as novel plant protectants

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### Introduction

Strong public and Federal mandates have supported the development of safer, more ecologically friendly pesticides that have fewer non-target effects, but still are able to provide a high degree of plant protection at an affordable price. The use of seed treatments has been especially effective in protecting seeds germinating in infested soil conditions and in recent years they have grown in favor of other inundative approaches to pest management of soil-borne diseases (Ragsdale, *et al.* 1993). Seed treatments are favored because of reduced and localized amounts of active ingredients that protect the plant particularly well during its vulnerable early stages of growth.

Chemical seed treatments have worked very effectively to provide a safe protective zone in the developing rhizosphere around the germinating seed, thus delaying or preventing pathogen infection of the young seedling. There are numerous commercially available synthetic pesticides available for use by growers, but some of these compounds are highly toxic or expensive making them less desirable. Additional concerns are that several of the currently commercially available seed treating compounds will not be re-registered for continued use because of significant environmental and safety concerns. Because of these concerns there is a growing demand for new and improved seed protectants to fill this void and this supports the development and testing of new antimicrobial compounds (Ragsdale, *et al.* 1993, Wedge, 2005).

Many natural plant-derived chemicals have proven antimicrobial/pesticidal properties including the coumarins (1,2-benzopyrone) found in a variety of plants such as clover, sweet woodruff and grasses (Hoult & Paya 1996). Preliminary research has shown the coumarins to be a highly active group of molecules with a wide range of antimicrobial activity against both fungi and bacteria. Previous studies have shown that those coumarins possessing hydroxy or ether functional groups were most biologically active and it is believed that coumarins may be functioning as natural defense molecules within the plant (Hussain, *et al.* 2003, Khan *et al.* 2004). One group of well-known hydroxylated coumarin derivatives are the phytoalexins which possess complex chemical structures and serve as a first response defense molecule in many plants (Hammerschmidt 1999).

Within this study, cyclic coumarin molecules will serve as a starting point for exploration of new derivative compounds which possess a range of antimicrobial activity to be used as seed protectants against soil-borne diseases. Coumarins in their own right are bioactive, are very inexpensive and they react readily in chemical reactions to generate a variety of structures making them ideal substrates for the synthesis generation of new coumarin derivatives (Hussain, *et al.* 2003, Khan, *et al.* 2004). In order to explore fungicidal

improvements of the coumarin molecule, several derivative compounds were synthesized using 4-hydroxy-coumarin (Dittmer, 2005). Halogenated forms of coumarin were of particular interest due to antimicrobial activity found in previous studies (Laas, *et al.* 2006). The synthesized halogenated coumarin compounds included iodinated, brominated and chlorinated forms and these compounds were then screened for antifungal activity and compared against the original 4-hydroxy-coumarin compound. In addition, plant phytotoxicity screening was conducted on all compounds to determine their impact on soybean seed germination and development. Results of these screening studies provide valuable insights into optimization and improvements of the coumarin chemical structure for improved plant protection activity.

Within this study three halogenated coumarin-derived compounds were synthesized and evaluated for antifungal activity against three economically important and taxonomically diverse soil-borne plant pathogenic fungi: *Macrophomina phaseolina* (causal agent of charcoal rot) which infects seedlings early, but actual disease symptoms develop later in the plant's life after plant stress, *Phytophthora* spp. (causal agent of seedling damping off and root rot) a water mold that can infect and destroy seedlings and root systems early in the growing season and throughout the plant's life and *Pythium* spp. (causal agent of seedling blight) another water mold that can infect and devastate seeds and young seedlings primarily in the early growing season. All of these soil-borne disease-causing fungi enter the root tissue of the soybean plant, and young seedlings are especially susceptible. This makes these fungi ideal for the purposes of screening bioactive coumarin derivatives and evaluating them as potential seed protectants.

**Materials and methods**

**Chemical synthesis** - Organic compounds were synthesized using 4-hydroxy-coumarin purchased from Sigma Chemical Company, St. Louis, Missouri and three unique compounds were synthesized.

**Seed treatment supplies** - Commercial Magnacoat seed treatment polymer was donated by Gustafson LLC, Plano, Texas. Soybean seed was provided by Dr. James Long, Kansas State University, Southeast Agricultural Research Center, Parsons, Kansas. All seed tests were conducted with Northrup King S49q9 Round-Up Ready Soybean variety.

**Fungal cultivation** - Fungal isolates of *M. phaseolina*, *Phytophthora* spp. and *Pythium* spp. were cultured on 0.5 × potato dextrose agar (Difco PDA) at 25C under full-spectrum lights. These cultures were allowed to grow and mature for 7-10 days prior to assays.

**Fungal assays** - *In vitro* anti-fungal assays consisted of screening each compound as an overlay resulting in a final concentration of 1000 µg/ml. All compounds were dissolved in methanol, and a methanol-only and an untreated control were used to monitor any methanol effects within the assay. In addition, 4-hydroxy-coumarin at 1000 µg/ml was used as an internal positive control to gauge the efficacy of the coumarin modified derivatives. Petri plates (10 × 50 mm) containing 6 ml of half strength PDA fungal growth media were used for the bioassay. A concentrated stock of each synthesized derivative was over-laid on top of this growth media using a sterile bent glass rod. Screened compounds were allowed to diffuse into the media for one hour prior to fungal inoculation. Fungal plugs 6 mm in diameter were cut from the leading edge of the fungal culture plates and placed culture side down in the middle of the assay plates. These plates were then incubated for 14 days at

25°C under continuous full-spectrum light. Growth was measured from the edge of the inoculation plug every other day. All treatments were done in triplicate and the experiment was repeated. Data were analyzed statistically using SAS ANOVA test for variance with an alpha level of 0.05.

**Phytotoxicity assays** – Magnacoat - only treated, Magnacoat with coumarin derivatives incorporated at 1000 µg/ml and untreated soybean seed were used to study the impact of coumarin derivatives on soybean seed germination and early development. Following manufacturer's recommendations on seed polymer treatment protocol (Gustafson, LLC), soybean seeds were treated and spread on wax paper to dry for 12 hours prior to germination tests. Each *in vitro* germination test consisted of 20 soybean seeds of each treatment group, wrapped in four layers of damp paper towels and sealed in re-sealable plastic bags. Seeds were grown for eight days at 26°C and germination data were recorded as a percentage of seed tested. Treated seeds were also observed daily for any changes in coloration (chlorosis, necrosis, etc.), size and rate of growth (overall length and internode lengths), and root development (primary and lateral) as compared to the untreated and Magnacoat - only treated control seedlings. All germination tests were repeated and results were analyzed statistically using SAS ANOVA test for variance with an alpha level of 0.05.

**Results**

Halogenated coumarin compounds have as high or higher anti-fungal activity when compared to 4-hydroxy-coumarin alone. All of the halogenated coumarin compounds had good solubility in methanol and when used in the fungal assays against *Macrophomina phaseolina*, *Pythium* spp. and *Phytophthora* spp. growth, the brominated, chlorinated and iodinated coumarin compounds had statistically significant antifungal activity against these soil-borne fungi. After 14 days of growth, the brominated, chlorinated and iodinated coumarins consistently displayed 100% fungal inhibition and notably these compounds sustained this high level of inhibition even after 21 days growth within the assay. The 4-hydroxy-coumarin control also displayed no decrease in activity over time in these assays. The methanol control did not display any significant fungal inhibition and the fungus grew to the limits of the methanol and untreated control plate margins within three days.

Preliminary soybean phytotoxicity tests were completely negative, with no brominated, chlorinated or iodinated coumarin derivatives affecting either seed germination or seedling development as compared to untreated and Magnacoat - only treated seeds.

**Discussion**

Results of this study suggest that halogenated coumarin compounds are extremely active against a broad range of soil-borne fungi. The fungi used in these bioassays are quite diverse in respect to their taxonomy/phylogeny with both *Pythium* spp. and *Phytophthora* spp. representing the more primitive Oomycetes and *Macrophomina* a more advanced Coelomycete. All of these fungi cause economically important diseases on a number of crops. *Macrophomina* has been exceptionally difficult to manage as a mid-season disease on more than 500 different species of plants, and the halogenated coumarins may offer one unique strategy for managing this disease causing fungus.

The consistent level of high antifungal activity, coupled with the chemical stability of the halogenated coumarins make these compounds very attractive for further experimentation. Future research will include additional fungal inhibition assays of other plant pathogenic fungi to determine the range of targeted activity and also extensive plant assays on a variety of crop plants to determine if any phytotoxicity levels of these compounds exist. The results of this study offer compelling evidence for further studies and highlight the economic and industrial importance of halogenated coumarin derivatives as natural plant protectants.

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## Total synthesis of (±)-rocaglamide and its analogue

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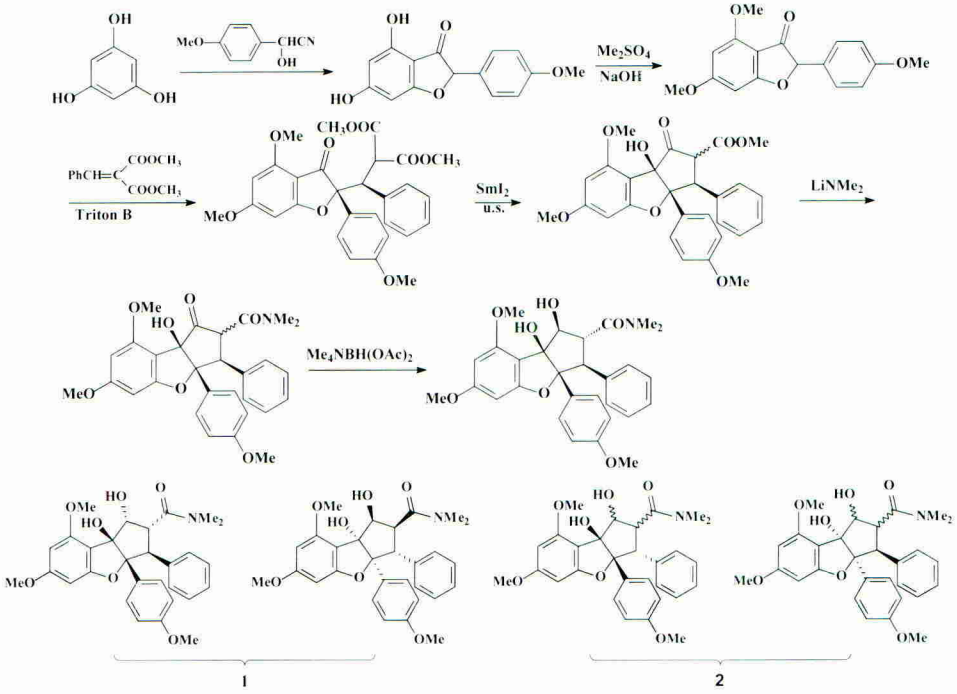
Rocaglamide, featuring a cyclopenta[*b*]benzofuran ring system, was isolated from *Aglaia elliptofolia* and its structure determined by Ming in 1982. Subsequently, rocaglamide and its analogues were found to exhibit diverse biological activities such as anti-leukemic, anticancer and insecticidal effects. Both the structural complexity of rocaglamide and its significant biological activity make it an attractive synthetic target. The tricyclic core structure bearing a highly functionalized cyclopentane moiety with five stereogenic centers also presents a considerable challenge. Herein we report a concise route to (±)-rocaglamide (1) and its racemic analogue (2) schemed below.




Preliminary bioassay showed that both 1 and 2 were good repellents, but 1 exhibited much stronger insecticidal activity than 2. The results are listed in Tables 1 and 2.

Table 1. Activity of 1 and 2 as repellents to *Plutella xylostella*

| Compd. | Conc. µg/mL | Efficiency % | Compd | Conc. µg/mL | Efficiency % | Compd | Conc. µg/mL | Efficiency % |
|---|---|---|---|---|---|---|---|---|
| | | 80.0 | | | 60.0 | | | 60.0 |
| | 200 | 0 | | 200 | 77.8 | | 200 | 77.8 |
| | | 20.0 | 1 | | 77.8 | 2 | | 75.0 |
| Azadirachtin | Average | 33.3 | | Average | 71.9 | | Average | 70.9 |
| | | 40.0 | | | 55.6 | | | 80.0 |
| | 100 | 60.0 | | 100 | 80.0 | | 100 | 60.0 |
| | | 20.0 | | | 55.6 | | | 40.0 |
| | Average | 40.0 | | Average | 63.7 | | Average | 60.0 |

Table 2. Insecticidal activity of 1 and 2 (% mortality)

| Compd. | Conc. µg/mL | *Pieris rapae* | *Plutella xylostella* | *Laphygma exigua* | *Helicoverpa armigera* |
|---|---|---|---|---|---|
| 1 | 200 | 86.7 | 90.0 | 100 | 88.1 |
| | 100 | 60.0 | 46.7 | 100 | 74.1 |
| 2 | 200 | 66.7 | 80.0 | 50.1 | 69.3 |
| | 100 | 13.3 | 33.3 | 11.5 | 14.8 |
| Azadirachtin | 200 | 60.0 | 83.3 | 96.2 | 48.1 |
| | 100 | 46.6 | 66.7 | 96.2 | 37.0 |
| Control | | 0 | 0 | 13.3 | 10.0 |

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# Spirotetramat - discovery, chemistry and physicochemical properties

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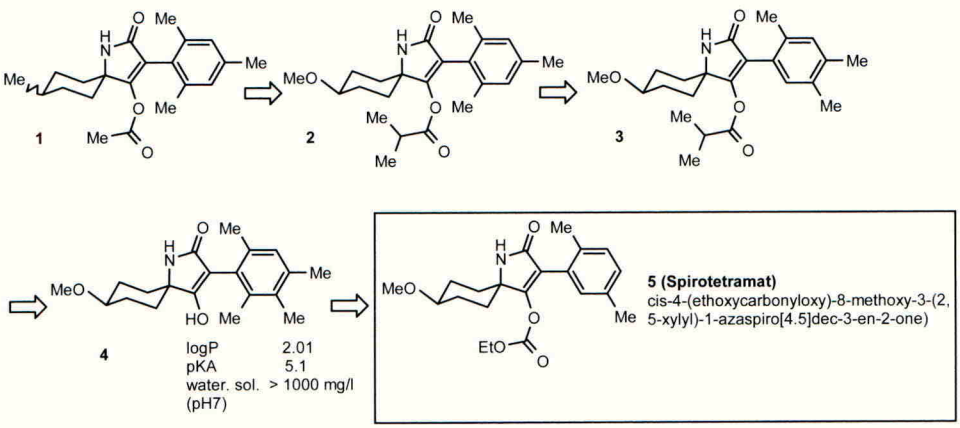
**Abstract**
The discovery and synthesis of spirotetramat as the first broad spectrum phloem mobile insecticide against sucking pests will be described. The physicochemical properties of the compound and its corresponding enole will be given. Spirotetramat is the third promising development compound of the cyclic ketoenole family. In comparison to spirodiclofen and spiromesifen, spirotetramat shows a two-way systemicity after foliar application. Due to this unique behavior a broad spectrum of sucking pests can be controlled with good residual efficacy.

The two broad spectrum acaricides spirodiclofen (trade names Envidor, Daniemon and Sinawi) and spiromesifen, which shows excellent activity against whiteflies, (trade name: Oberon) belong to the new chemical class of tetronic acid derivatives discovered at Bayer CropScience during the 1990s. Spirodiclofen and spiromesifen have a new mode of action (interference of lipid biosynthesis) and show no cross-resistance to resistant mite or whitefly field populations (Bretschneider *et al.*, 2003).

**Discovery of spirotetramat**
In parallel to the discovery of acaricidally active tetronic acid derivatives, attempts were made to improve the acaricidal as well as the herbicidal efficacy in the chemical class of tetramic acid derivatives. Starting with 1-amino-4-methyl-cyclohexanecarboxylic acid methyl ester, prepared via a Bucherer-Bergs reaction (Munday, 1961), the tetramic acid derivative 1 was synthesized. A significant improvement of the herbicidal efficacy compared to the unsubstituted spirocyclic analogues was found. In addition, an excellent acaricidal performance in the case of 1 and surprisingly moderate to good efficacy against the green peach aphid *Myzus persicae* (MYZUPE) was found.

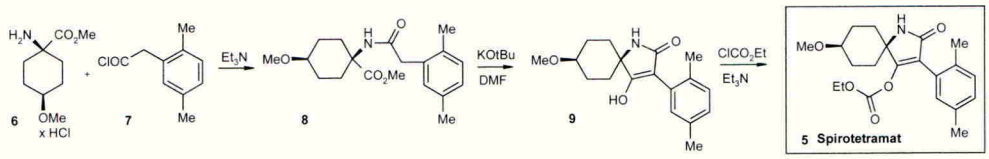
Further evaluation led to the *cis*-methoxy substituted spirocyclic tetramic acid derivative 2 which showed very good control of MYZUPE, but also severe phytotoxicity. After more intensive work, a 2,4,5-trimethyl phenyl group 3 was introduced instead of the mesitylene moiety and these showed a broad aphicidal efficacy and an improved crop selectivity. In addition, good whitefly control was observed. Inhibition of ACCase as the mode of action for the tetramic acids and a two-way systemicity (phloem and xylem mobility) was proved with the 2,3,4,6-tetramethyl-phenyl enole 4. An improvement of the aphicidal activity together with the preservation of the favorable plant selectivity was achieved.

At the end of a fine tuning process lasting about 10 years, spirotetramat 5 (Andersch *et al.*, 1998) was selected as adevelopment candidate, with a good efficacy in field trials after foliar application against sucking insects such as aphids, psyllids, scales, mealy bugs, whiteflies, thrips and root aphids. Because of the systemic properties, new shoots are also protected.

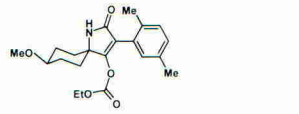


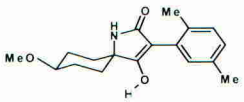

**Synthesis of spirotetramat**

Spirotetramat 5 can be synthesized in a twelve step convergent synthesis. The first key intermediate is the *cis*-4-methoxy-1-aminocyclohexanecarboxylic acid methyl ester × HCl 6 which is synthesized in a five step sequence starting with the hydrogenation of 4-hydroxy-anisole to 4-methoxycyclohexanone followed by a Bucherer-Bergs reaction to form a mixture of isomers of the corresponding hydantoin. After separation of the *cis*-isomer the hydantoin is hydrolyzed to the amino acid which is esterified with thionylchloride/methanol to the methylester 6 (Fischer *et al.*, 2002). The second key intermediate 2,5-dimethyl-phenylacetyl chloride 7 can be synthesized in a four step straight forward route starting from *p*-xylene (Himmler, 2005). Acylation of 6 with 7 leads to the phenylacetyl-aminoester 8. After a Dieckmann-condensation with KO*t*Bu the spirotetramat-enole 9 is formed which is finally acylated with ethyl-chloroformate to yield spirotetramat 5.



## Physicochemical properties



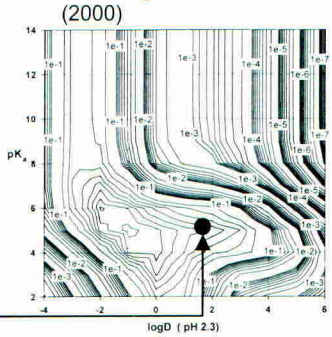
**Spirotetramat**

LogD 2,7 (pH 2,3)
pKa none
water solubility 30 ppm (pH7)
mp. 142 °C

LogD 1,7 (pH 2,3)
pKa 5.2
water solubility 2700 ppm (pH7)
mp. 226 °C

**Spirotetramat-enole**

Model calculation of mass transported in the phloem according to Satchivi *et al.* (2000)

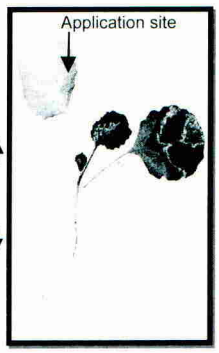



## Two-way systemicity of spirotetramat-enole

**Two-way systemic properties**

**Acropetal movement (xylem and phloem)**

**Basipetal movement (phloem)**




Translocation of a.i. equivalents of [$^{14}$C] spirotetramat applied as a SC 240 formulation to cabbage leaves in combination with rape oil methylester (0.1%). Two droplets of 5 µl (0.4 µg a.i.) were applied onto the 1st true leaf 2 days prior to analysis


## Conclusions

Because of its physicochemical properties the spirotetramat-enole fulfilled the requirements for a phloem systemic pesticide according to the model calculation (Satchivi *et al.*, 2000). In comparison to spirodiclofen (LogP 5.83) and spiromesifen (LogP 4.55) which remain on the leaf surface spirotetramat (LogD 2.7) penetrates the leaves and is distributed in the plant via the spirotetramat-enole. New leaves and roots are protected.

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